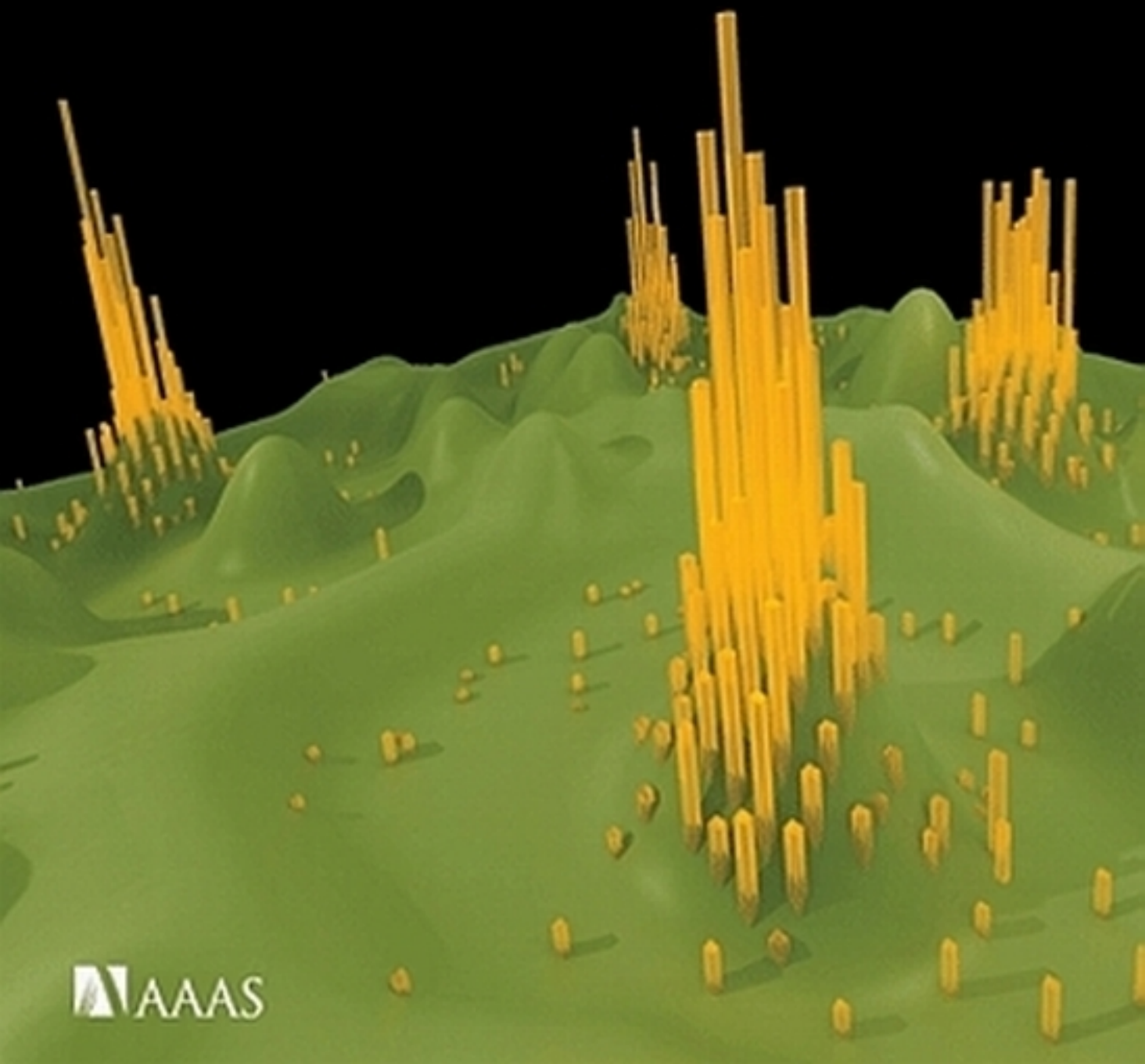
24 September 2010 | $10

Science



AAAS

EDITORIAL

1573 Science for Physicians
Molly Cooke

NEWS OF THE WEEK

1582 A Way to Heal Science Education, But Is There the Political Will?
1583 Misconduct by Postdocs Leads to Retraction of Papers
1585 Genomics Researchers Upset by Rivals' Publicity
1586 Origin of Most Deadly Human Malaria Comes Out of the Mist
1587 From *Science*'s Online Daily News Site
1588 Long-Fought Compromise Reached on European Animal Rules
1589 A Tricky Balance Between Activists' and Researchers' Rights
1590 New Type of Cosmic Dust Tells of Galaxy's Violent History
>> *Report p. 1622*
1591 Parlous Times for Seed Banks Spell Trouble for Australian Agriculture
1591 From the *Science* Policy Blog

NEWS FOCUS

1592 Home, Home Outside the Range?
>> Science *Podcast*
1595 Mouse Studies Challenge Rare Immune Cell's Powers
1596 Behind the Eco-Label, a Debate Over Antarctic Toothfish

LETTERS

1598 Retraction
L. B. Buck
A Recount of Tropical Tree Species
J. Ghazoul
Science Standards: Averages Deceive
A. DePristo
Science Standards: Value the Teachers
S. B. Oppenheimer
Response
A. I. Leshner et al.
Education Framework Needs Better Foundation
F. J. Rutherford
1599 CORRECTIONS AND CLARIFICATIONS

BOOKS *ET AL.*

1600 The Evolution of Language
W. T. Fitch;
The Evolution of Human Language
R. K. Larson et al., *Eds., reviewed by N. J. Enfield*
1601 Worst Case Bioethics
G. J. Annas, reviewed by T. May

POLICY FORUM

1603 Family Planning and the Millennium Development Goals
W. Cates Jr. et al.

PERSPECTIVES

1604 More Supernova Surprises
J. M. Laming
>> *Report p. 1624*
1605 The Rise of Sunflowers
T. Stuessy
>> *Brevia p. 1621*
1606 A Bit of Texas in Florida
C. Packer
>> *Report p. 1641*
1607 Exposing a DUX Tale
M. S. Mahadevan
>> *Report p. 1650*
1609 STM Ready for the Time Domain
M. Morgenstern
>> *Report p. 1628*
1610 Branching Takes Nerve
J. R. Rock and B. L. M. Hogan
>> *Report p. 1645*
1611 Dendrites Do It in Sequences
A. Destexhe
>> *Report p. 1671*

SCIENCE PRIZE ESSAY

1613 Immunology for Clinicians: A "Trojan Horse" Approach
C. M. Gray et al.

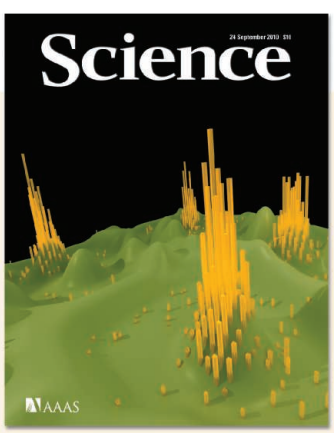
CONTENTS continued >>



page 1592



pages 1605 & 1621



COVER

Overlay of a scanning tunneling microscope image of four iron-copper dimers adsorbed on a surface (green) with a snapshot of their electron-spin dynamics (yellow spikes). On page 1628, Loth *et al*. describe the measurement of electron spin relaxation times of individual atoms extracted from a time series of tunneling current measurements with nanosecond time resolution.

Image: Sebastian Loth, IBM Research/Almaden

DEPARTMENTS

1569 This Week in *Science*
1575 Editors' Choice
1578 *Science* Staff
1581 Random Samples
1615 AAAS News & Notes
1676 New Products
1677 *Science* Careers

Published by AAAS

REVIEW

1616 Reaction-Diffusion Model as a Framework for Understanding Biological Pattern Formation
S. Kondo and T. Miura

BREVIA

1621 Eocene Patagonia Fossils of the Daisy Family
V. D. Barreda et al.
Fossil evidence suggests that daisies and sunflowers may have originated in South America more than 47 million years ago.
>> *Perspective p. 1605*

REPORTS

1622 The Ubiquity of Micrometer-Sized Dust Grains in the Dense Interstellar Medium
L. Pagani et al.
The light scattered by small dust grains can tell us about the properties of star-forming regions in our Galaxy.
>> *News story p. 1590*

1624 Observing Supernova 1987A with the Refurbished Hubble Space Telescope
K. France et al.
Images of the remnants of a stellar explosion reveal details of fast astrophysical shock waves.
>> *Perspective p. 1604*

1628 Measurement of Fast Electron Spin Relaxation Times with Atomic Resolution
S. Loth et al.
Scanning tunneling microscopy is used to monitor the fast relaxation dynamics of an atomic spin adsorbed on a surface.
>> *Perspective p. 1609*

1630 Optical Clocks and Relativity
C. W. Chou et al.
The tiny relativistic effects of everyday life can be measured by clocks ticking at optical frequencies.
>> Science *Podcast*

1633 Alkali-Stabilized Pt-OH_x Species Catalyze Low-Temperature Water-Gas Shift Reactions
Y. Zhai et al.
The formation of hydrogen from carbon monoxide and water is catalyzed by the formation of oxidized platinum atoms.

1637 Graphene Double-Layer Capacitor with ac Line-Filtering Performance
J. R. Miller et al.
The performance of electrolytic capacitors in filtering circuitry was improved with high-surface-area graphene electrodes.

1639 Slip Systems in $MgSiO_3$ Post-Perovskite: Implications for *D″* Anisotropy
L. Miyagi et al.
The major mineral phase in the lower mantle deforms preferentially along one lattice plane.

1641 Genetic Restoration of the Florida Panther
W. E. Johnson et al.
Recovery of the Florida panther population is attributed to the benefits of admixture with panthers from Texas.
>> *Perspective p. 1606*

1645 Parasympathetic Innervation Maintains Epithelial Progenitor Cells During Salivary Organogenesis
S. M. Knox et al.
Peripheral nerves maintain the undifferentiated state of epithelial progenitor cells during development.
>> *Perspective p. 1610*

1647 Olfactory Plasticity Is Regulated by Pheromonal Signaling in *Caenorhabditis elegans*
K. Yamada et al.
A nematode odor response is regulated by population density through dauer pheromone, a neuropeptide, and neprilysin peptidase.

1650 A Unifying Genetic Model for Facioscapulohumeral Muscular Dystrophy
R. J. L. F. Lemmers et al.
Sequence variants shared by patients with a genetically complex form of muscular dystrophy explain how the disease arises.
>> *Perspective p. 1607*

1653 The Genetic and Molecular Basis for Sunscreen Biosynthesis in Cyanobacteria
E. P. Balskus and C. T. Walsh
A gene cluster encodes a four-enzyme pathway that uses an unusual mechanism to synthesize small-molecule sunscreens.

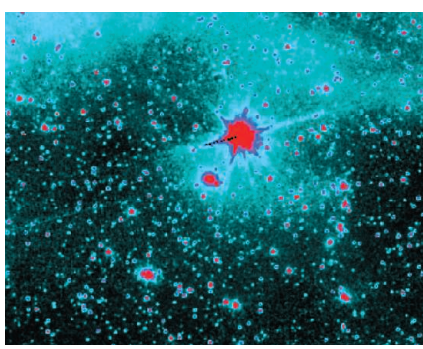
1656 A General Mechanism for Network-Dosage Compensation in Gene Circuits
M. Acar et al.
A simple gene circuit consisting of an activator and an inhibitor is insensitive to the number of copies of a gene network.

1660 A *Vibrio* Effector Protein Is an Inositol Phosphatase and Disrupts Host Cell Membrane Integrity
C. A. Broberg et al.
Altering the homeostasis of membrane-bound signaling molecules allows a bacterial pathogen to corrupt cell function.

1663 α-Synuclein Promotes SNARE-Complex Assembly in Vivo and in Vitro
J. Burré et al.
A protein implicated in neurodegeneration promotes the assembly of membrane fusion complexes.



pages 1606 & 1641



pages 1590 & 1622

1667 Stability of the Regulatory T Cell Lineage in Vivo
Y. P. Rubtsov et al.
A subset of T cells that suppress immune-mediated inflammation is maintained by self-renewal.

1671 Dendritic Discrimination of Temporal Input Sequences in Cortical Neurons
T. Branco et al.
Dendrites of neurons are sensitive to the sequence of synaptic activation and can implement cortical computation.
>> *Perspective p. 1611*

CONTENTS continued >>

SCIENCEONLINE

SCIENCEXPRESS
www.sciencexpress.org

Channel-Mediated Tonic GABA Release from Glia
S. Lee et al.
The neurotransmitter GABA is tonically released from cells through an anion channel with an unusually large pore.
10.1126/science.1184334

Intestinal Stem Cell Replacement Follows a Pattern of Neutral Drift
C. Lopez-Garcia et al.
Intestinal stem cells form an equipotent population where loss of a stem cell is compensated by multiplication of a neighbor.
10.1126/science.1196236

Lineage Relationship Analysis of RORγt$^+$ Innate Lymphoid Cells
S. Sawa et al.
Immune cells develop to preempt intestinal colonization by microbial symbionts.
10.1126/science.1194597

Particle Acceleration on Megaparsec Scales in a Merging Galaxy Cluster
R. J. van Weeren et al.
Observations show that shocks within the intracluster medium are capable of producing extremely energetic cosmic rays.
10.1126/science.1194293

Magnitude of the 2010 Gulf of Mexico Oil Leak
T. J. Crone and M. Tolstoy
Modeling videos suggest that around 4.4 million barrels of oil escaped from the broken *Deepwater Horizon* well.
10.1126/science.1195840

SCIENCENOW
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Highlights From Our Daily News Coverage

Tuning In to Highest Energy Cosmic Rays
A chance observation suggests that radio waves reflected off Antarctic ice could be used to track particles from space.

Metal Smasher Makes Aluminum as Strong as Steel
A new technique could produce a cheap alternative to titanium for aircraft and body armor.

Is Narcissism Good for Business?
Narcissists' ideas aren't any more creative, but narcissists help get things done.

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RESEARCH ARTICLE: Synthetic Lethal Screen of an EGFR-Centered Network to Improve Targeted Therapies
I. Astsaturov et al.
A targeted RNAi screen reveals potential targets for combination approaches to cancer treatment.

RESEARCH ARTICLE: Regulation of Ras Localization by Acylation Enables a Mode of Intracellular Signal Propagation
A. Lorentzen et al.
Active Ras is relayed between subcellular compartments by the acylation cycle.

PERSPECTIVE: The Myelin Brake—When Enough Is Enough
W. B. Macklin
The lipid phosphatase PTEN and the scaffold protein Dlg1 restrict myelination in the peripheral nervous system.

NETWATCH: Peptidome
Deposit experimental mass spectrometry data into a public repository; in Technical Information.

NETWATCH: DNASU Plasmid Repository
Browse and contribute to a repository that archives and distributes plasmids; in Technical Information.

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Making Each Other More Human
V. Venkatraman
The 50-year partnership of Don and Ada Olins is based on good communication and a shared passion for chromatin structure.

Don't Worry, I'm (Un)Professional: A Guide to Your Laboratory Colleagues
A. Ruben
Our Experimental Error columnist asks, "Who are the people in your fume hood?"

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REVIEW: Long Synthetic Peptides for the Production of Vaccines and Drugs—A Technological Platform Coming of Age
G. Corradin et al.
Improved methods for producing long synthetic peptides, together with bioinformatics approaches, offer promise for developing clinically relevant products.



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Rational combination therapy.

RESEARCH ARTICLE: A Naturally Occurring Human Minidysferlin Protein Repairs Sarcolemmal Lesions in a Mouse Model of Dysferlinopathy
M. Krahn et al.
Gene therapy by mini-gene transfer may be useful for treating patients with certain progressive muscular dystrophies.

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Science for Physicians

TENSION OVER THE PLACE OF THE BASIC SCIENCES HAS BEEN A HALLMARK OF MEDICAL EDUCATION in the United States for more than 100 years. In 1910, the Carnegie Foundation for the Advancement of Teaching issued *Medical Education in the United States and Canada*. Known as the Flexner report, it recommended devoting the first 2 of the 4 years of medical school to teaching the fundamentals of disciplines such as anatomy, chemistry, physiology, and pathology. This report served as the foundation for important improvements in medical education that lasted until the 1970s. Since then, U.S. medical schools have built on this foundation, with curricula in years 1 and 2 that increasingly aim to better integrate the science that underlies medicine with clinical practice. This path has proven successful, but there remains a lack of consensus on how much exposure to the basic sciences physicians in training need, with some even arguing that a background in science is not needed at all. For this and other reasons, my colleagues and I have recently completed an intensive 4-year study of U.S. medical education. We conclude that science will be critical for the future physician and that preparing physicians to incorporate science and scientific advances over their careers should be a central goal of medical education at all levels.



A basic science education establishes a foundational understanding on which medical practice is, or should be, based. Effective patient care increasingly requires that physicians keep pace with rapidly evolving technologies and treatments, continually assimilating a vast amount of new and complex information. Indeed, studies have shown that both experienced and novice physicians form more coherent, durable, and flexible understandings of diseases and their treatments when they can link conditions to basic science concepts.

But there is an even more compelling reason to make basic science education essential for all physicians: stimulating curiosity and creating the scientific habits of mind that are essential for continual learning. Basic science research is a portal to the next generation of medical care. Thus, it is critical that both medical students and residents gain experience in critically assessing and interpreting research, not just in terms of outcomes and clinical effectiveness, but also in the context of biological plausibility and mechanisms. In addition, physicians and physicians-to-be must become familiar with those emerging areas of biomedical science with a potential to affect patient care. Under what circumstances, for example, will personal genome sequences become important for patient care, and what problems and opportunities will they create for a physician?

Engagement with science also must extend far beyond the first 2 years of medical school in order to develop "the intellectual flexibility on which adaptive expertise depends."* Despite the increased attempts at integration in recent decades, science remains too sequestered in the curriculum, and passive instructional approaches focus on what is already known at the expense of engaging learners in what needs to be discovered. Teaching must emphasize not only today's knowledge, but also the methods and paths of reasoning that led to it. Even more important is a focus on what we don't know, in anticipation of unfolding areas of knowledge. Rather than avoid topics in which uncertainty prevails, teachers of clinical medicine need to emphasize the important areas of incomplete knowledge about the condition that a patient has, as well as the scientific disciplines and approaches that will be required to improve diagnosis and treatment.

The real issue is not why basic science, or how much of it, is needed during formal medical education. Instead, the question is how this science is to be much more effectively taught, both throughout the entire 7- to 10-year period of medical school, internship, and residency, and in the continuing education required of physicians. **– Molly Cooke**

10.1126/science.1197542

*M. Cooke, D. M. Irby, B. C. O'Brien, *Educating Physicians: A Call for Reform of Medical School and Residency* (Jossey-Bass, San Francisco, 2010).

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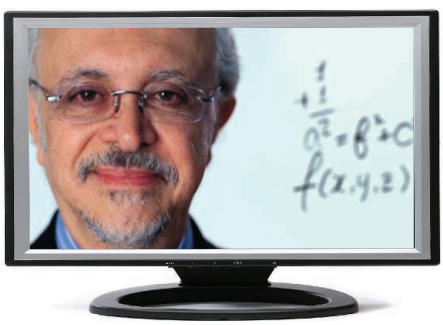


Malaria's origins 1586

SCIENCE IN U.S. SCHOOLS

A Way to Heal Science Education, But Is There the Political Will?

The Obama Administration received bold advice last week from two presidentially appointed bodies on how to improve math and science education in U.S. elementary and secondary schools. The reports focus on different aspects of the problem, but they share one important theme: The nation can't produce the scientifically literate workforce it needs unless everyone—elected officials, scientists, the private sector, and the public—pays more attention to the topic. Science education advocates have already embraced that message, but implementing it will be much more difficult.



Memories. PCAST's Mario Molina and others recall their favorite teacher (http://bit.ly/pcastvideo).

The heftier report—both in scope and potential clout—comes from the President's Council of Advisors on Science and Technology (http://bit.ly/pcast-on-stem). Despite its pedestrian title, *Prepare and Inspire: K-12 Education in Science, Technology, Engineering, and Math (STEM) for America's Future*, the PCAST report offers a soaring vision of how to achieve President Barack Obama's goal to lift U.S. students by the end of the decade "from the middle to the top of the pack" in the rankings on international tests. The other report (http://bit.ly/nsb-on-innovators), by the policymaking body for the National Science Foundation, says the country needs to be more concerned about the high end of the student population, namely, those most likely "to become leading STEM professionals and perhaps the creators of significant breakthroughs in scientific and technological understanding."

The challenge is stark, says the PCAST report: "[T]oo many American students conclude early in their education that STEM subjects are boring, too difficult, or unwelcoming, leaving them ill-prepared to meet the challenges that will face their generation, their country, and the world." So the panel's solutions—which include a new federal agency to promote digital learning, higher salaries for the cream of the nation's teaching corps, and the creation of 1000 STEM-focused schools—must be up to that challenge, say PCAST members Eric Lander and S. James Gates Jr., who co-chaired the 19-member panel. Lander is director of the Broad Institute in Cambridge, Massachusetts, and Gates is a professor of physics at the University of Maryland, College Park.



As the worm turns. Kindergarten students in San Francisco study earthworms with help from a visiting scientist.

The cost of implementing those reforms could approach $1 billion a year, according to the report, but Lander says anything less risks falling short of meeting the president's goal. "It isn't about the money. We're handing over to the president a set of ideas that we think make a lot of sense. And now they have to be worked."

That's where things are likely to get sticky, says Grover "Russ" Whitehurst, director of the Brown Center on Education Policy at the Brookings Institution in Washington, D.C. Lander says "the vast majority of what we are talking about will not require new money because it is either already something that could be covered under existing authorities and appropriations, or under existing appropriations with new authorities." But Whitehurst, who led the Department of Education's (ED's) research arm, the Institute for Education Sciences, during the Bush Administration, says that's wishful thinking. "PCAST has some useful recommendations in the abstract, but they are not grounded in the realities of politics, legislation, or how federal agencies operate," he says.

Going through the report's key suggestions, Whitehurst explains how much heavy lifting would be required to implement each one. "The program to pay bonuses to master science teachers would require specific congressional authorization," he says about the report's suggestion to give a $15,000-a-year salary boost to some 22,000 teachers around the country. "The funding that PCAST mentions is from something in the Administration's 2011 budget, which is unlikely to be funded at anything like the requested levels. STEM schools would certainly require a new authorization and appropriation. The 21st century after-school program is a formula-funded grant that is to be used by statute to improve student performance in the core academic subjects. I don't see any authority in legislation to allow ED to focus it on STEM."

Whitehurst is also skeptical that the Obama Administration has sufficient political capital to achieve major structural changes, in particular a $200-million-a-year entity to promote new educational technologies and digital learning materials. "An Advanced Research Projects Agency–Education seems like a good idea," says Whitehurst, "but it, too, would have to be authorized and funded if it is to have the scale to address the goals articulated in PCAST. ARPA–ED can't exist unless Congress wants it and wants to spend real money on it."

Key congressional education leaders declined comment on the PCAST report, saying they needed time to digest it. And although the idea of boosting STEM education enjoys bipartisan support, Republicans

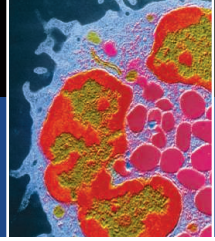
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Should we move species to save them?
1592



Reassessing what basophils do
1595

have sharply criticized the tens of billions more in overall federal spending on education during the Obama Administration. They also take a dim view of expanding the federal bureaucracy and giving Washington a bigger role in an area that constitutionally is the province of state and local governments.

One area in which the federal government is now almost invisible is nurturing the smartest students. "Talent development takes a lot of hard work," says Camilla Benbow, who led a National Science Board panel that last week issued its report on what it labels "STEM innovators." Benbow says most education reformers "have been focused on trying to bring up the mean." The new report, in contrast, aims "to increase the top level of achievement by focusing on excellence as well as equity." Its recommendations include portable, merit-based scholarships for middle- and high-school students along the lines of NSF's prestigious graduate research fellowships. "We don't want talent to be dependent on who your parents are, or where you were born," says Benbow, an educational psychologist at Vanderbilt University in Nashville, Tennessee. **–JEFFREY MERVIS**

SCIENTIFIC MISCONDUCT

Misconduct by Postdocs Leads to Retraction of Papers

Last week, Mount Sinai School of Medicine in New York City said that two postdoctoral fellows working in the lab of noted gene-therapy researcher Savio Woo had been fired for research misconduct. In a statement, the school said that Woo had been cleared of any wrongdoing in an internal investigation and had voluntarily retracted four research papers. Other researchers say the impact of the retractions probably won't be widespread, but they are an unfortunate blow for a distinguished scientist in the latter stages of his career.

"He's one of the leaders in gene therapy. He's widely respected and well-liked," says Michele Calos, a gene-therapy researcher at Stanford University in Palo Alto, California.

Mount Sinai spokesperson Ian Michaels declined to name the postdocs, but the process of elimination suggests they are Li Chen and Zhiyu Li, each the first author on two retracted papers and the only ones not currently listed in the Mount Sinai directory as faculty members. The statement (available at http://tinyurl.com/wooretraction) says the school has reported its findings to the appropriate federal agencies.

The retracted papers focus on two areas of gene-therapy research. One pair describes a method for using a bacteriophage enzyme to introduce therapeutic genes. In 2005, Chen and Woo reported in the *Proceedings of the National Academy of Sciences* (*PNAS*) that mice with the metabolic disorder phenylketonuria (PKU) appeared to be cured using this technique (*Science*, 17 June 2005, p. 1736). A 2008 paper in *Human Gene Therapy* described the method's use in human cells.

The work is conceptually similar to a method pioneered by Calos and colleagues a few years earlier. They demonstrated that a bacteriophage enzyme called phiC31 integrase could be used to introduce a gene at specific spots in the genome—an improvement over virus-based methods of gene delivery, which tend to insert genes at random locations. Chen and Woo used the same approach with a more recently discovered phage integrase called phiBT1. Calos recalls being surprised when their *PNAS* paper came out because her lab had also been experimenting with phiBT1 but without much success. "It's not as efficient or as precise as phiC31," Calos says. "It was kind of a dud." Calos says she told Woo about their unpublished findings and expressed concern that something must be wrong with both papers. Initially, Woo defended the work, Calos says, but he must have developed doubts.



Savio Woo. An investigation cleared him of wrongdoing.

Yuet Wai Kan of the University of California, San Francisco, who communicated Woo's paper to *PNAS* as a member of the National Academy of Sciences, says the retraction probably won't be a huge setback for the field. "The Calos system is more popular" with other researchers, Kan says.

In retraction letters sent to both journals, Woo merely cites "data irregularities." Two subsequent papers published in 2007 in *Molecular Therapy* by Chen and Woo appear to build on the phiBT1 work, but they have not been retracted.

The other two retracted papers deal with an entirely different topic. Published in 2008 in the *Journal of the National Cancer Institute* (*JNCI*) and in 2009 in *Human Gene Therapy*, they involve using genetically engineered bacteria to fight cancer. The origins of the idea date back nearly 2 centuries to observations that cancer sometimes regresses in patients who become infected with certain bacteria. More recently, researchers have discovered that some bacteria appear to concentrate in tumors and kill them. "The excitement about this is that you can get this specific accumulation in tumors that you don't see with other treatments," says Neil Forbes, who studies cancer-fighting bacteria at the University of Massachusetts, Amherst.

One limitation is that the immune system can prevent bacteria from dispersing throughout a tumor. In the *JNCI* paper, Li, Woo, and colleagues showed that adding an inflammation-suppressing gene to *Clostridium perfringens* bacteria enhanced its ability to kill tumors in mice. In the *Human Gene Therapy* paper, they describe knocking out a *Clostridium* toxin gene, which could reduce the side effects of treatment. In retraction letters, Woo wrote that some micrographs in the two papers were duplicated.

The problems with that work may be more serious, however. Forbes is writing a review article, and he says he contacted Woo to ask if it would still be appropriate to cite the retracted *JNCI* paper as proof of the concept that engineering bacteria with immune-suppressing genes could enhance their tumor-fighting capacity. Woo promptly wrote back urging him not to. Even so, Forbes says he doesn't expect any major setbacks for the field given the timely retraction of the papers.

–GREG MILLER

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SCIENTIFIC PUBLISHING

Genomics Researchers Upset by Rivals' Publicity

Last week was a tough one for Stephan Schuster. A genomicist at Pennsylvania State University, University Park, Schuster has pioneered genome-sequencing techniques for both modern and ancient DNA. But over the course of 2 days, announcements by rival teams upstaged two of his current projects. On 15 September, the U.S. Department of Agriculture (USDA) and the chocolate giant Mars Inc. announced in a press release that they had sequenced the cacao tree, whose seeds are the source for chocolate. The very next day, Elizabeth Murchison of the Sanger Institute in Hinxton, U.K., announced at a conference in Tasmania that her group has sequenced the genome of the Tasmanian devil, a talk widely publicized by Sanger and the sequencer manufacturer Illumina Inc. in press releases.

For Schuster, the back-to-back announcements were devastating. He is part of separate groups sequencing both genomes. One group has already submitted a paper on the cacao genome, and the other is currently analyzing the Tasmanian devil sequence. "With what happened yesterday, I don't believe in scientific publication anymore," says Schuster, who thinks work shouldn't be publicized until after peer review. "We tried to be a good citizen, … and we lost."

His view may be a minority one in the rapid-fire world of genome sequencing. The precedent for announcing genome sequences to the media well in advance of publication was established a decade ago, when rival sequencers held a press conference with President Bill Clinton to herald the completion of drafts of the human genome. Papers came out 8 months later (*Science*, 30 June 2000, p. 2304; 16 February 2001, p. 1177). And although some complain about science by press release, others say it's okay. "It's hard to fault people for putting out a press release," says Daniel Rokhsar of the Joint Genome Institute in Walnut Creek, California. "When people have put a lot of effort into doing these genomes, they want to make sure they are on the map."

Murchison picked a good spot to get on the map: She announced her group's sequence in Hobart, Tasmania, at a regional genomics meeting, AMATA 2010. Since 1996, the numbers of this carnivorous marsupial have plummeted by more than 80% because of a deadly transmissible cancer that seems unstoppable, fueling fears of extinction and inspiring a mad scramble by researchers and conservationists to save the species (*Science*, 18 February 2005, p. 1035). Murchison didn't give out many details about the new sequence but said that with new technology, the genome took just 2 months to sequence. "We have a reference genome that we are happy with," she says.

Schuster and the Penn State group, working with Vanessa Hayes, now at the J. Craig Venter Institute in San Diego, California, had sequenced and assembled a draft of the genome of one devil, with 8X coverage. The team had also lightly sequenced the genome of another devil from the opposite side of Tasmania. By comparing the two genomes, the researchers identified single-base differences, called SNPs, that they used to help assess the diversity of these animals across Tasmania. These assessments are helping conservationists who are trying to raise devils in captivity representing the full diversity of the species, says Hayes. She and Schuster are planning to publish the genome when the population-structure studies are completed; until then, putting out a press release or statements about the project would be premature, they argue.



Scooped. Stephan Schuster's rivals beat him to the punch last week with announcements about the Tasmanian devil (*top right*) and cacao genomes prior to publication.

While Schuster was fretting about his group's Tasmanian devil work being overshadowed, his colleagues working on the cacao genome were scrambling to prevent the same thing from happening to their project. The rival Mars-USDA collaboration announced that it had assembled the sequence and opened a Web site to provide other researchers access to the data.

The Penn State researchers are part of a group led by Claire Lanuad of CIRAD in Montpellier, France. The group, which is still revising its cacao genome manuscript, countered the Mars-USDA announcement by posting a summary of its results on a preprint Web site called *Nature Precedings* and issuing press releases of its own. "We were waiting to talk about our paper [after] it was published," says Mark Guiltinan, the plant molecular biologist shepherding the cacao project at Penn State. "But we would like people to know about our project."

The Penn State-CIRAD group sequenced the Criollo variety of cacao, assembling 76% of the cacao genome into its 10 chromosomes and placing 82% of the 28,798 genes along this DNA. "We were at the point of the [Mars-USDA] press release about 6 months ago," claims Guiltinan.

USDA's David Kuhn, based at the Agricultural Research Service in Miami, Florida, says his team is still gathering sequence data, but he disagrees with Guiltinan's assessment of where the Mars-USDA cacao genome stands. "Our assembly is so much better than theirs, it's astounding," he says. Kuhn's group, which sequenced a different variety, says it has assembled 92% of the genome and identified 35,000 genes, 97% of which have been validated. (It is unclear if the two varieties of cacao have different numbers of genes.) "Cacao is a well-behaved genome," he explains. The assembly "went better than we could have imagined."

None of these projects has stood the test of peer review, but the headline grabbing is unlikely to cloud the prospects for publication. "As long as the paper contains new insights that are not in the press release, then there is minimal [negative] impact," says Richard Gibbs, director of the Human Genome Sequencing Center at Baylor College of Medicine in Houston, Texas, and an editor at *Genome Research*. *Science* editor Laura Zahn agrees: The novelty—a criterion for acceptance by journals such as *Science* and *Nature*—lies not in the genome itself but in the analysis. Last week's announcements indicate that the race to publish is in the home stretch.

–ELIZABETH PENNISI

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PARASITOLOGY

Origin of Most Deadly Human Malaria Comes Out of the Mist

It's a gorilla!

In the search for the origin of the parasite that causes the most severe malaria in humans, researchers have implicated relatives in a variety of species. Last year, new evidence appeared to have solved the mystery: Chimpanzees, it seemed clear, harbored the ancestor to human *Plasmodium falciparum*, which causes raging fevers in a few hundred million people each year, killing about 1 million. But now a new study of DNA plucked from the feces of a huge number of African apes has led the small but impassioned malaria origins field to do a double take, as it appears that the closest relative to human *P. falciparum* is a parasite found only in gorillas.

A research team led by virologist Beatrice Hahn of the University of Alabama, Birmingham, used the polymerase chain reaction to amplify *Plasmodium* DNA from a whopping 2739 fecal samples from chimpanzees, gorillas, and bonobos. As Hahn's team explains in the 23 September issue of *Nature*, bonobos were free of the parasite, but 473 of the samples from chimpanzees and gorillas tested positive. When they compared the DNA sequences in evolutionary trees, the chimpanzee sequences of what's known as *P. reichenowi*, fingered last year as the human *P. falciparum* predecessor, clustered together, as expected. But surprisingly, none showed a close relationship to human *P. falciparum*. A gorilla branch of the *Plasmodium* family tree neatly included all human *P. falciparum* sequences. (The nomenclature of the new sequences has yet to be sorted out, and for now they are simply referred to as gorilla *P. falciparum*.)

The evidence has persuaded researchers who have studied the question for years, including those who have put forward different hypotheses. "This provides the clearest picture yet," says Richard Carter, a malaria geneticist at the University of Edinburgh. "It looks definitive."

Karen Day, a malaria geneticist at New York University's Langone Medical Center in New York City, notes the critical difference between this study and those that preceded it is the number of samples analyzed. "By the greater depth of sampling, we're getting closer to the truth," says Day.

Although the findings may have little practical importance other than to re-emphasize that pathogens can jump from apes to humans, it has stoked a hot debate back to life about both the origin of human *P. falciparum* and the timing of its emergence.

Until last year, scientists had isolated only one sample of the chimpanzee parasite, *P. reichenowi*. Then a group led by Nathan Wolfe of Stanford University in Palo Alto, California, and Stephen Rich of the University of Massachusetts, Amherst, reported online 3 August in the *Proceedings of the National Academy of Sciences* (*PNAS*) that they had fished eight new *P. reichenowi* isolates from the tissue and blood of wild chimpanzees that had died in Côte d'Ivoire or lived in a Cameroonian sanctuary. Working with evolutionary biologist Francisco Ayala of the University of California (UC), Irvine, the team contended that a sequence analysis clearly showed how human *P. falciparum* evolved from *P. reichenowi.*

This finding challenged a theory that *P. reichenowi* and *P. falciparum* had evolved separately when humans and chimpanzees split from a common ancestor some 6 million years ago. It also added weight to an earlier assertion by Ayala and Rich, called Malaria's Eve hypothesis, that all extant strains of *P. falciparum* came from a single ancestor and that the parasite had established itself in humans perhaps as recently as 10,000 years ago.

A subsequent *PNAS* paper published online 19 January by a French team collaborating with Ayala further backed the story that the chimp parasite gave rise to the human one. The group, led by geneticist Franck Prugnolle of the Institute of Research for Development in Montpellier, France, retrieved DNA sequences of the malaria parasites from ape fecal samples for the first time. They obtained five good samples from chimps and, surprisingly, seven very different sequences from gorillas. They noted that the gorilla *P. falciparum* could be a reservoir for *P. falciparum* in humans, but *P. reichenowi* still appeared to be a closer relative.

Then came Hahn's work suggesting the opposite. Ayala says he is convinced that Hahn's team has it right. "It's very impressive," says Ayala. "This does not invalidate most of the previous work but adds considerable new information, and the picture changes in a constructive way." Specifically, he would like to analyze mutations in the gorilla sequences to see if they can help clarify the evolutionary history of *P. falciparum.*

Hahn, who specializes in the origin of the AIDS epidemic, ventured into malaria after she read the Prugnolle study and learned that *Plasmodium* DNA could be extracted from feces. She was studying the impact of the chimpanzee AIDS virus, SIVcpz, in the Gombe community in Tanzania, which she



Big surprise. Gorillas, not chimpanzees, harbor what looks like the ancestor to human *P. falciparum*.

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believed was leading to their premature deaths. "We wanted to make sure the chimps were killed by the virus and not something else," says Hahn. "Obviously, SIVcpz and malaria is worse than malaria alone." But her team didn't find any malaria in the 400 or so fecal samples of Gombe chimps they studied.

Still, Hahn was puzzled that Prugnolle found the malaria parasite in gorillas, which seemed to put a question mark on the purported ancestral relationship between *P. reichenowi* and *P. falciparum*. So she collaborated with ape researchers across Africa to obtain fecal samples and reopen what seemed to be a closed case.

Hahn stresses that the new data leave open the date of the jump from gorillas to humans. And she's less sanguine than Ayala about the new sequences answering the question. *Plasmodium* does not mutate much, she explains, making it difficult to calibrate a so-called molecular clock that enables evolutionary biologists to back-calculate the timing of a pathogen moving from one species to another. "Short of finding fossils, I don't know how to do it," says Hahn.

Ajit Varki, an evolutionary biologist at UC San Diego, agrees, but he believes a human gene mutation, *CMAH*, which arose about 2 million years ago, may provide a clue to how and when the jump occurred. The mutation changes sialic acid receptors on red blood cells, rendering them much less susceptible to *P. reichenowi*, and Varki suspects that the gorilla *P. falciparum* similarly would have great difficulty infecting human cells. "If I put that *falciparum* from gorillas into humans, I'd bet it wouldn't make them sick," he says. He contends that the bug made the jump just one time because the barriers are so high. And it likely required a powerful force like the advent of agriculture some 10,000 years ago, which benefited the mosquito vector and created close living conditions for the parasite to have a chance to pass through several humans and adapt to its new host.

Michael Worobey, an evolutionary biologist who has also focused on the origin of the AIDS epidemic, is similarly intrigued that gorilla *P. falciparum* has not repeatedly infected humans. But he, too, is convinced that it's the ancestor to human *P. falciparum*, noting that it's 10 times closer than the chimp's *P. reichenowi*. "For years, the field has been stumbling around in the dark, and now the light switch has gone on and … it's really quite clear," Worobey says. And he tips his hat to Hahn, an outsider to the field. Says Worobey: "A virus person now cleaned up 20 years of *Plasmodium* research."

–JON COHEN

CREDITS (TOP TO BOTTOM): CERIDWEN FRASER, UNIVERSITY OF SYDNEY; KATRIEN KOLENBERG

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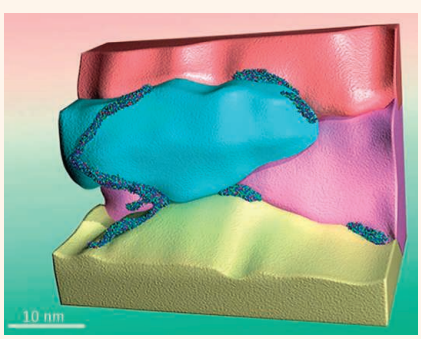
Hitchhiking on Kelp

You're not from around here, are you? That's what marine biologist Ceridwen Fraser thought when she spotted a piece of seaweed encrusted with large barnacles on a New Zealand beach in 2009. Turns out she was right. Genetic data reveal that the kelp came from islands hundreds of kilometers to the south. And it carried other hitchhikers—at least 10 types of marine invertebrates, including two tiny crustaceans, a sea spider, six species of mollusks, and a sea star. The find, reported online in the *Proceedings of the Royal Society B*, represents the first direct evidence that whole communities of organisms can make long ocean voyages floating on organic rafts. http://bit.ly/kelp-raft



Steely Strong Aluminum

Snuffing out a cigarette butt with a 10-ton boot would be excessive, but using the equivalent on certain metals can yield amazing results. By squeezing an aluminum alloy between two anvils with a force of about 60,000 kilograms per square centimeter, researchers have created a metal that's as strong as steel but much lighter.



Hierarchy. Under pressure, zinc and magnesium atoms (small dots) clustered together by size.

In the new study, an international team of materials scientists subjected an alloy of aluminum called aluminum 7075 (which contains small percentages of magnesium and zinc) to a metal-processing technique called high-pressure torsion (HPT). Basically, HPT involves clamping a thin disk of metal to a cylindrical anvil and pressing it against another anvil, all while turning one anvil slowly. The metal attained a strength of 1 gigapascal, the researchers report in *Nature Communications*. That's equal to some of the strongest steels and more than three times higher than conventional aluminum.

When the team examined samples using a technique called atom probe tomography, they found that HPT had deformed the lattice of atoms into what the researchers call a hierarchical nanostructure: The size of the aluminum grains was reduced, and the zinc and magnesium atoms clustered together in groups of various sizes, depending on whether they were located inside the aluminum grains or on the edges. http://bit.ly/strong-stuff

Dance Your Ph.D. Finalists Chosen

The dreaded question: "So, what's your Ph.D. research about?" You could bore them with an explanation. Or you could dance. That's the idea behind "Dance Your Ph.D." Over the past 3 years, scientists from around the world have teamed up to create dance videos based on their graduate research. This year's contest, launched in June by *Science*, received 45 brave submissions.



Last week, judges—including scientists, choreographers, and past winners—announced the finalists in four categories: physics, chemistry, biology, and social sciences. Each receives $500. The overall winner will be announced at the Imagine Science Film Festival in New York City on 19 October. View the finalists and runners-up at http://bit.ly/dance-PhD

Read the full postings, comments, and more at **http://news.sciencemag.org/sciencenow**.

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Watchful eyes. New E.U. rules allow research on nonhuman primates under strict ethical oversight.

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ANIMAL RESEARCH

Long-Fought Compromise Reached On European Animal Rules

After a decade-long tug of war over new rules governing animal experiments in the European Union, both research and animal-welfare advocates say they can live with the final legislation approved this month. Following a flurry of intense lobbying from all sides, the European Parliament gave its final approval on 8 September to a new directive spelling out rules governing animal research in academic and industrial labs.

As with most E.U. legislation, however, the directive will take effect through laws enacted by each member country. And that, say observers and animal-rights activists, will determine how much the directive will improve animal welfare and change the way researchers work with animals. "This is not the end but the start of a process," says Sonja Van Tichelen, director of Eurogroup for Animals, an animal-welfare organization based in Brussels. The 25 E.U. member countries have more than 2 years, until January 2013, to adopt laws that put the directive's rules into effect.

The rules cover research with all vertebrates and extend coverage for the first time to cephalopods, which include octopi and squid. Unlike the previous rules, which stipulated only that animal research be authorized by a competent authority, the new directive requires that all research using such animals must pass an ethical evaluation that takes into account possible alternatives and refinements that could improve the welfare of the animal subjects.

The directive also sets out for the first time minimum housing and care standards for dozens of the most common animals used in research—a step that many praise as a significant advance for animal welfare across the European Union. Each European breeding, supply, or research establishment must have an "animal welfare body" that is supposed to ensure that the rules are obeyed.

The previous E.U. rules on animal research date from 1986, and officials have been working on the new regulations since 2001. Animal-welfare groups had pushed hard for a phaseout of all research on nonhuman primates (*Science*, 14 November 2008, p. 1037). The final rules do ban the use of great apes, with a few exceptions related to saving a species from extinction or an unexpected outbreak of severe disease in people. They also allow research with other primate species, including baboons, marmosets, and macaques if "there is scientific justification" that no alternative is available. After a transition period, the use of wild-caught primates is to be phased out, and only captive-born primates that are two or more generations removed from wild-caught animals are allowed to be used.

For most scientists in the European Union's biggest research countries, "it's pretty much going to be business as usual," says Wendy Higgins, spokesperson for the Humane Society International in Nottingham, U.K., because those countries already have fairly strict animal-protection laws. Stefan Treue, director of the German Primate Center in Göttingen, agrees. "The type of research that I do is so highly regulated by German law that I don't think it will change much with the directive," he says. "From that perspective, I can live with it." But that doesn't mean he's completely satisfied with the final version. Treue faults the directive for banning certain research without spelling out an ethical justification. "It just draws a political line: There are first-tier animals and second-tier animals." That allows politicians to say they banned ape research, he says, but it is not a sound ethical basis for deciding whether certain experiments are justified. He says he would have preferred a rule that said, simply, "for every experiment, we have to weigh the benefits against the suffering of the animals."

In addition to primates, the regulations include special rules for "domestic species," notes Treue. The use of feral or stray cats or dogs is generally prohibited, and all cats, dogs, and primates must have a record of their veterinary and social history and all projects in which they have been used.

Another aspect that researchers will be watching carefully is a new requirement to publish nontechnical summaries of all animal-research projects. There is some worry that information will turn into a tool for activists who want to prevent all animal research, says Thorsten Ruppert, spokesperson for the German Association of Research-based Pharmaceutical Companies. Depending on how the database is organized, he says, it could help activists target specific institutes or researchers.

The directive also divides invasive procedures into discomfort severity categories of mild, moderate, and severe, with different rules for each. Exactly how those terms are translated into law in different countries could influence certain kinds of research, Treue notes. "There is no way to foresee how countries will interpret this," he says. Researchers who study chronic pain, certain types of cancer, or arthritis, for example, could be affected, he says. Cornelia Exner, a member of the Commission on Animal Protection and Experimentation for the German funding agency DFG, agrees. "A tumor is painful, not only for humans but also for animals," she says. To comply

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with the new rules, she says, "in some cases the experimental protocols will have to be modified to minimize pain and suffering in the animals."

The greatest impact of the directive will likely be on the newer E.U. members in Eastern Europe. In some member countries, animal-housing standards, for example, were regulated by voluntary guidelines instead of enforceable law, Higgins says.

In Romania, says Mircea Leabu, a cell biologist at the "Carol Davila" University of Medicine and Pharmacy in Bucharest and a member of the National Committee of Scientific Research Ethics, many of the new standards have been adopted as the E.U. discussion moved forward. And because animal research is expensive, he says, Romanian scientists are already motivated to use as few animals as possible. But the new rules boost control and enforcement measures, he says, and may require more people qualified to evaluate the ethical aspects of animal experiments. Indeed, enforcement measures are one of the most important areas to watch as the directive is translated into national laws, Higgins says.

Observers agree that standardized rules across Europe will be a step forward for both researchers and animal welfare. "I think it's wonderful. That's a core task of the E.U.," Treue says. Higgins agrees. "The new law brings in a number of new and welcome measures," she says. "The sooner that new legislation is implemented the better."

–GRETCHEN VOGEL

ANIMAL-RIGHTS ACTIVISM

A Tricky Balance Between Activists' and Researchers' Rights

In 2004, animal-rights activists broke into psychology laboratories at the University of Iowa. A video they recorded, available on YouTube, shows several people wearing gloves and ski masks smashing computers and other equipment with hammers, spray-painting slogans on the wall, and loading rats into plastic tubs. Hundreds of animals were taken, and the damage totaled more than $400,000. A few days later, the Animal Liberation Front claimed responsibility.

Last November, just as the statute of limitations was about to expire, federal prosecutors charged Scott DeMuth, a sociology graduate student at the University of Minnesota, Twin Cities, with felony conspiracy in connection with the break-in (*Science*, 18 December 2009, p. 1609). DeMuth's trial was scheduled to begin last week, but at the last minute prosecutors offered him a plea agreement: They would drop all charges related to the Iowa case if he pleaded guilty to a lesser misdemeanor conspiracy charge for an unspecified role in a 2006 break-in at a ferret-breeding facility in Minnesota. DeMuth took the deal and now faces up to 6 months in jail and a fine of up to $5000, considerably lighter penalties than he could have received if convicted of the felony.

DeMuth's case and others highlight the difficulty of identifying the small minority of animal-rights activists who break the law and successfully prosecuting those who are charged. In July, a federal judge in San Jose, California, threw out a felony indictment against activists accused of harassing researchers at the University of California (UC) campuses in Berkeley and Santa Cruz. They had been indicted under the federal Animal Enterprise Terrorism Act (AETA), a 2006 law intended to crack down on animal-rights extremists. Several incidents of vandalism and harassment aimed at scientists at UC Los Angeles (*Science*, 21 December 2007, p. 1856) and elsewhere remain unsolved.

In DeMuth's case, it's not clear what evidence prosecutors had. "I don't have any idea what the government says he actually did in terms of Iowa," says his lawyer, Michael Deutsch. The indictment says only that he conspired "to intentionally damage and cause the loss of property." According to Deutsch, in a pretrial hearing last week prosecutors said they planned to introduce entries from DeMuth's personal diary and MySpace page as evidence of his animal-rights sympathies, as well as expert testimony that his height (somewhat below average at 5'6") matches that of an individual in the video. Deutsch says that DeMuth, who was 17 at the time, was asleep in his parents' home in Minnesota the night of the incident. "His mother was going to come in and testify," he says. The U.S. attorney's office in Davenport, Iowa, where the case was to be tried, declined to comment.



Plea deal. Scott DeMuth pleaded guilty to a misdemeanor charge after prosecutors dropped charges related to a lab break-in at the University of Iowa.

DeMuth and his supporters have argued that his indictment was punishment for refusing to cooperate with a grand jury investigating the Iowa break-in. His research focuses on radical activist groups, and he claimed that forcing him to break confidentiality agreements with his subjects to reveal anything he might know about the incident would violate his academic freedom. Two activists and friends of DeMuth's said they would go to jail for contempt of court rather than testify against him if his case went to trial—a major factor, Deutsch says, in his decision to accept the plea deal. DeMuth did not respond to a request for comment.

In California, federal prosecutors have had a similarly difficult time. In July, Judge Ronald Whyte dismissed an indictment against four activists for threatening and intimidating researchers at UC Berkeley and UC Santa Cruz in 2007 and 2008. Supporting documents describe several instances of people chanting and chalking slogans on the sidewalk outside researchers' homes; an incident in which activists allegedly tried to force their way into a researcher's home; and the distribution of flyers around town with the names and addresses of researchers, who were described as "murderers and torturers." The indictment made no mention of more dramatic events in Santa Cruz, including an attempted arson at a scientist's home (*Science*, 8 August 2008, p. 755).

Whyte said the indictment failed to provide sufficient evidence that the activists' actions constituted "true threats" as opposed to political protest protected by the Constitution. However, in dismissing the case "without prejudice," Whyte left the door open for prosecutors to try again. Jack Gillund, a spokesperson for the U.S. Attorney's office in San Francisco, said he could neither confirm nor deny plans to reindict. However, Robert Bloom, the lawyer for one of the defendants, says his client recently received and complied with a warrant seeking a DNA sample, which seems to suggest the government still has an interest in the case.

The Santa Cruz activists were the first to be charged under AETA, the 2006 law that increased possible penalties for those convicted of crimes committed in the name of animal rights. (DeMuth was origi- ▶

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nally charged under AETA, but because the two break-ins happened before the law was enacted, prosecutors later reindicted him using language from the older Animal Enterprise Protection Act.) Subsequently, two men were convicted under AETA for releasing 650 minks and vandalizing a mink ranch in Utah in 2008. Most recently, in July, authorities charged a Utah man under AETA for lighting a fire that destroyed a sheepskin factory in Denver in 2009.

AETA's track record—two convictions in 4 years—raises questions about whether it's having its intended effect. Frankie Trull, president of the Foundation for Biomedical Research (FBR) in Washington, D.C., says AETA has given investigators new tools to monitor communications by people planning attacks and use such evidence in court. But FBR's statistics on illegal activities by animal-rights activists show no clear trend since the law was enacted.

Some researchers who have been on the receiving end of harassment aren't sure AETA has had much impact. J. David Jentsch, a neuroscientist at UCLA whose car was set on fire in 2009, says activists still spew venom about him on their blogs and gather at his house about once a month to yell slogans that go well beyond free speech. "Any reasonable person would see it as threats," he says. Jentsch points out that many of the actions directed at him were illegal before AETA was enacted. "Blowing up my car was obviously illegal, but no one has been arrested," he says.

Activists loathe the law but for different reasons, says sociologist David Pellow, DeMuth's graduate adviser. The Santa Cruz case in particular has convinced some activists that authorities are using AETA to criminalize lawful protest, Pellow says. "Others have argued that this law may have the unintended consequence of driving people in precisely the wrong direction and sending them underground," he says.

The reason so few extremists who commit these crimes have been caught, Pellow says, is that they often operate in very small, autonomous groups. "There's a cell of maybe three or four people who trust each other … who get together to do these actions," Pellow says. Each cell is independent, so if one is infiltrated or someone snitches, other cells are unaffected. Pellow says, "They are very clever and very good at practicing a security culture."

–GREG MILLER

ASTRONOMY

New Type of Cosmic Dust Tells of Galaxy's Violent History

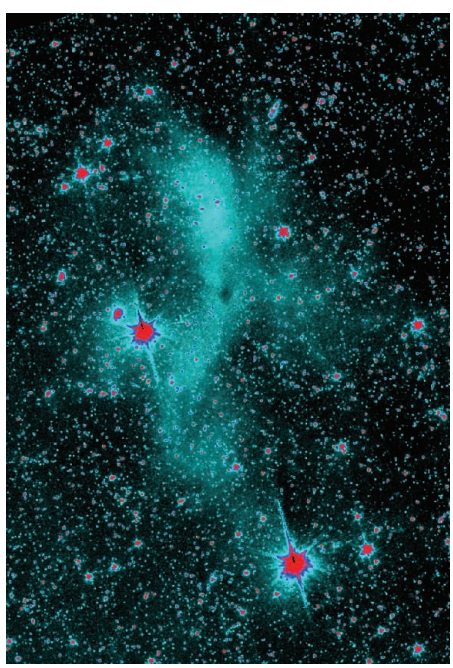
The vast interstellar expanses of galaxies are filled with clouds of dust and gas in which new stars and planets form. The physical details of these cocoons have been hazy to astronomers. Now, researchers have found that the cores of many of these clouds are swarming with dust grains 10 times as large as those previously detected. These grains scatter starlight to produce a so-called coreshine effect, which the researchers say could be used to probe the age and history of interstellar clouds.

The team, led by Laurent Pagani of the Paris Observatory and Jürgen Steinacker of the Max Planck Institute for Astronomy in Heidelberg, Germany, describes the results on page 1622. "I think this is an exciting new approach to understanding the structure within molecular clouds," says Doug Johnstone, an astronomer at the NRC-Herzberg Institute of Astrophysics in Victoria, Canada, who was not involved in the research.

Interstellar clouds generally appear as dark patches against the starry sky because their dust absorbs and scatters the light from stars behind them. But light at infrared wavelengths—0.8 micrometers (microns) and longer—can filter through most interstellar dust, whose average grain size is about 0.1 micrometers in radius. That's why researchers use infrared wavelengths to image stars and galaxies that would otherwise be obscured by dust clouds.

Pagani and colleagues originally set out to measure the dust in a prominent cloud in the Milky Way called L183. Looking at images from the Spitzer Space Telescope's Infrared Array Camera, the researchers made a surprising find: The densest parts of the cloud appeared to glow at a midinfrared wavelength far too long to shine through ordinary-sized dust.

"The only way to explain what we were seeing was with grains much bigger than usually thought, that is, grains of 1 micron size on average," Pagani says. Theorists had proposed that smaller dust grains could merge to form grains that large, but this was the first direct evidence of them.



Glowing. Researchers saw diffuse light in the dense cores of dust clouds.

CREDIT: L. PAGANI *ET AL.*, *SCIENCE*

Studying 110 dust cloud cores surveyed by Spitzer, Pagani and colleagues detected coreshine in about half of them. The researchers say large grains—and the consequent coreshine—may be a marker of a cloud's history. They speculate, for instance, that the Gum/Vela nebula lacks coreshine because most of its agglomerated large grains might have been smashed back to smithereens by a supernova explosion a million years ago.

Coreshine measurements could be used not only to determine the structure and density of dust clouds but also to estimate their age, Pagani says. "It takes a while for the grains to grow by collision in between them, and the collisions are all the more frequent if the density is higher," he says. Using a model that relates growth speed to density, researchers can deduce from the quantity of coreshine "when the grains have started to grow and therefore the age of the cloud."

Impressed by coreshine's potential as a tool, Johnstone says he plans to calibrate and exploit the effect after the James Webb Space Telescope is launched 5 years from now. JWST—a 6.5-meter instrument designed for infrared observations—"should provide unprecedented images of the cloudshine feature with exquisite spatial resolution," Johnstone says. That faint glow will help astronomers look deeper into those hazy stellar birthing grounds.

–YUDHIJIT BHATTACHARJEE

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GENETIC RESOURCES

Parlous Times for Seed Banks Spell Trouble for Australian Agriculture

CANBERRA—Australia's seed banks are tumbling like dominoes, and the country's agricultural researchers and farmers may end up the losers. The Biloela Research Station in Queensland, home to a 60-year-old collection of seeds of legumes and livestock forage plants, is slated to shut down in the coming months. A plan is in place to rescue commercially valuable grain legumes. But the neglect of other prized accessions will make it harder for Australia to comply with a genetics resources treaty, which in turn could curtail access to overseas seed collections—a debilitating prospect for a country whose farms rely on imported species.

Until a few years ago, Australia's state governments, with agricultural R&D corporations, maintained six seed banks. The Grains Research and Development Corp. (GRDC) was the dominant contributor, but in 2008 it pared back its support to grains. As a result, in mid-2008 a bank in Adelaide holding Mediterranean forages such as alfalfa closed its doors; of its 45,000 accessions, 95% are held nowhere else in the world. Other seed banks are fighting for survival. According to Biloela curator Peter Lawrence, "Vital work to regenerate and conserve some globally unique plant genetic resources has stopped, and skilled staff have resigned or retired."

Now the Queensland government plans to shut down the Biloela bank as part of a "national strategy" to rationalize plant genetic resources, says Rex Williams, a science leader at Queensland's department of agriculture. Seed banks in Victoria and New South Wales have been approached to store some accessions. GRDC has pledged to pick up the tab over the next 4 years for commercial crop legumes. Accessions of forage plants, including ones that provide bush food for Aborigines, will be kept frozen. But without funds and skilled staff to periodically regenerate seeds, viable accessions will dwindle.

Like all gene banks, Biloela is obligated under the International Treaty on Plant Genetic Resources for Food and Agriculture to make seeds freely available on request. Funding shortfalls have made it increasingly difficult for Biloela and other seed banks to share seeds with partner nations, as required by agreements. That, says Adelaide bank curator Steve Hughes, risks serious repercussions for Australian agriculture—including loss of access to overseas varieties of wheat, Australia's most valuable crop.







Bitter harvest. Funding cuts imperil seed collections of purple clover (*top*), alfalfa (middle), and wild *Vigna* yams (*left*).

Many experts argue that the federal government, as treaty signatory, needs to step in to safeguard the collections. Seed banks "need long-term support that is outside grant or research support," says Megan Clarke, chief executive of CSIRO, Australia's national science agency and the country's main supporter of agricultural research. But as a stalemate over funding continues, Australia's plant genetic resources are looking increasingly fragile.

–ELIZABETH FINKEL

Elizabeth Finkel is a writer in Melbourne, Australia.

CREDITS (CLOCKWISE FROM LOWER LEFT): BOB LAWN/JAMES COOK UNIVERSITY; RICHARD SNOWBALL/AUSTRALIAN TRIFOLIUM GENETIC RESOURCE CENTRE, PERTH; STEVE HUGHES/SARDI

ScienceInsider

From the *Science* Policy Blog

Last week, a Senate panel heard about the **promise of human embryonic stem cell research** and the damage from last month's preliminary injunction. On 27 September, a federal appeals court will hear oral arguments in the suit brought by opponents. In the meantime, advocates lost a key congressional supporter when Representative Michael Castle (D–DE) lost his bid for a Senate seat. http://bit.ly/castle-loses

Energy Secretary Steven Chu pledged to **dismantle "the traditional stovepipes"** that confound research managers at the Department of Energy. But after 18 months on the job, Steven Koonin, his undersecretary for science, told an advisory board that bureaucratic turf-minding and red tape continue to hamper a series of research efforts involving supercomputing, material sciences, and fusion. http://bit.ly/KooninStoves

The general public favors **research with animals containing human tissue or genes** if it would help to improve human health and cure diseases, says a study by the Academy of Medical Sciences in the United Kingdom. The aim was to gauge public opinion on all types of such research for help in shaping policy guidelines due out next year. http://bit.ly/mixedGenes

A review board at the National Institutes of Health has called for **merging two NIH institutes** that study drug abuse and alcohol abuse. The recommendation comes 4 years after Congress asked NIH to create the board to think about ways to reorganize. NIH Director Francis Collins has expressed interest in the idea, but Congress is likely to have the final say. http://bit.ly/mergeNIH

The U.S. Senate has confirmed Nobelist **Carl Wieman as associate director for science** at the White House Office of Science and Technology Policy. The physicist is expected to spearhead the Administration's push to improve science education. Wieman joins Shere Abbott, Aneesh Chopra, and Philip Coyle in giving science adviser John Holdren a full complement of associate directors. http://bit.ly/WiemanIn

For more science policy news, visit **http://news.sciencemag.org/scienceinsider.**

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Home, Home Outside the Range?

Conservationists and ecologists are at odds over the wisdom of moving species threatened by climate change to new homes

HUAPING, CHINA—The stem of the ground-hugging orchid is bowed at the top, weighted down by five violet-tipped buds on the verge of blossoming. The swan's-neck shape gives the flower a demure look. Or perhaps it's just resigned to its fate: This is one of the last *Geodorum eulophioides* left on the planet.

The species is confined to a single hill behind a farmer's home in southwest China's Guangxi Province. Villagers "didn't know they had something so precious here," says Hong Liu, a conservation biologist at Florida International University and Fairchild Tropical Botanic Garden in Miami. But Guangxi is one of the world's nine orchid hot spots, and this patch of land where *G. eulophioides* resides is now part of Yachang Orchid Nature Reserve, a 220-square-kilometer territory with more than 130 orchid species. Liu and colleagues persuaded reserve managers in Huaping to give *G. eulophioides* some breathing space by fencing off the hill.

That action may also give scientists time to learn more about the rare orchid's biology. But it's unclear how long the species can hold out in the wild. Across China, climate change is nudging temperatures higher, disrupting rainfall patterns, and reducing the frequency of foggy days. Like the rest of northwestern Guangxi, Yachang suffered a serious drought last winter that forced rangers for the first time to pipe water into the heart of the reserve. And for the *G. eulophioides* on the reserve's edge, the human threat hovers, like a sword of Damocles, just outside the hill's chain-link fence.

In light of shifting climates and relentless development, scientists here are contemplating a controversial intervention: assisted colonization (AC). "Orchids will be severely affected by warming," says Feng Changlin, an ecologist at the Experimental Center of Tropical Forestry in Pingxiang. The idea is to move *G. eulophioides* and other acutely vulnerable orchids to new habitats that, as the world warms, become more suitable than present habitats—and hopefully, for a time, put them beyond harm's reach. But some scientists argue that such forced migration could do more harm than good.

One of the hottest debates in conservation biology these days is to what extent scientists should help embattled species cope with climate change. Not just orchids are at risk: All life forms, including our own, must adapt to climate change or dwindle and possibly perish. Scientists generally agree that first they should protect or shore up ecosystems, especially fragile ones such as cloud forests and coral reefs. Consensus breaks down, however, on what to do when a species can't keep pace with a changing world.

One camp insists that desperate times call for desperate measures. Habitat fragmentation caused by human activity has made it difficult or impossible for many species to migrate on their own to more suitable environments. Thus, a growing number of researchers argue that AC, also called managed relocation, is a vital conservation tool. "The future for many species and ecosystems is so bleak that assisted colonization might be their best chance," Ove Hoegh-Guldberg, director of the Global Change Institute at the University of Queensland in St. Lucia, Australia, and colleagues wrote in a clarion call for moving species in *Science* 2 years ago (18 July 2008, p. 345). "This is something that conservation organizations should and will start to do," says Chris Thomas, a conservation biologist at the University of York, U.K.

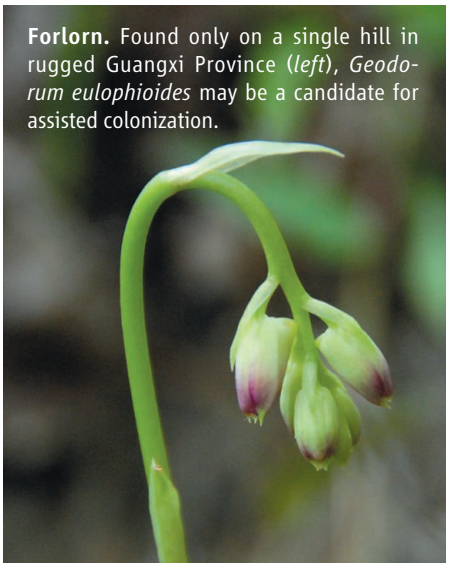
Other scientists worry that momentum for translocations is building too fast. "Advocates are not clear about what they are talking about. There are around 7 million species on Earth. Are they talking about moving them one at a time?" asks Daniel Simberloff, an éminence grise of conservation biology at the University of Tennessee, Knoxville. "There is no scientific basis to suggest that AC is the best alternative," adds Anthony Ricciardi, an invasive species biologist at McGill University in Montreal, Canada. AC, he warns, could interfere with habitat preservation and restoration and compete with such efforts

Online
sciencemag.org
Podcast interview with author Richard Stone.

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Forlorn. Found only on a single hill in rugged Guangxi Province (*left*), *Geodorum eulophioides* may be a candidate for assisted colonization.

for resources. "AC is nothing more than a techno-fix that may provide a temporary benefit in some cases but create new ecological problems in other cases. We cannot predict which outcome will occur," says Ricciardi, who, with Simberloff, laid out an indictment of AC in the September 2009 issue of *Trends in Ecology and Evolution*.

The dueling articles have ignited a spirited debate in journal opinion pages, university classrooms, and conferences, and agencies responsible for conservation are trying to figure out where they stand. Thomas believes that AC should be a higher priority than breeding threatened species in captivity. Others see AC as a last resort. "It's much better to help species to shift naturally whenever possible," says Stephen Willis, a conservation biologist at Durham University in the United Kingdom. Even backers call for caution. "Other options need to be carefully considered first and not discarded as if AC was a panacea," says Hoegh-Guldberg.

Butterfly effect

The world is replete with species that have been moved far beyond their native habitats. Consequently, "it is senseless to consider species distributions as somehow fixed and 'natural' and that the establishment and occurrence of a species elsewhere is therefore 'unnatural,' " says Thomas. Still, scientists on both sides of the AC debate are skittish because even a seemingly benign or beneficial introduction of a species to a new habitat can have disastrous consequences.

One cautionary tale, Thomas notes, is the cane toad, brought from Hawaii to Australia in 1935 to consume beetles that were ravaging sugar-cane crops. The toad apparently had little effect on the cane beetles—and it spread widely, becoming a pest in its own right. It makes a toxin blamed for steep declines in predators such as freshwater crocodiles and the western quoll, a ferocious marsupial cat. Australia is littered with that sort of ecological train wreck. "You only have to look at our history with invasive cacti, poisonous toads, and exploding rabbit populations to know that there are some real risks attached to assisted colonization," says Hoegh-Guldberg.

Bearing that in mind, he and his *Science* co-authors devised a risk-assessment scheme in which AC would be considered only after other options are ruled out. To be a colonization candidate, they proposed, a species must be at high risk of decline or extinction because of climate change; it would have to be feasible to move and establish the species elsewhere; and the benefits of relocation would have to outweigh the biological and socioeconomic costs.

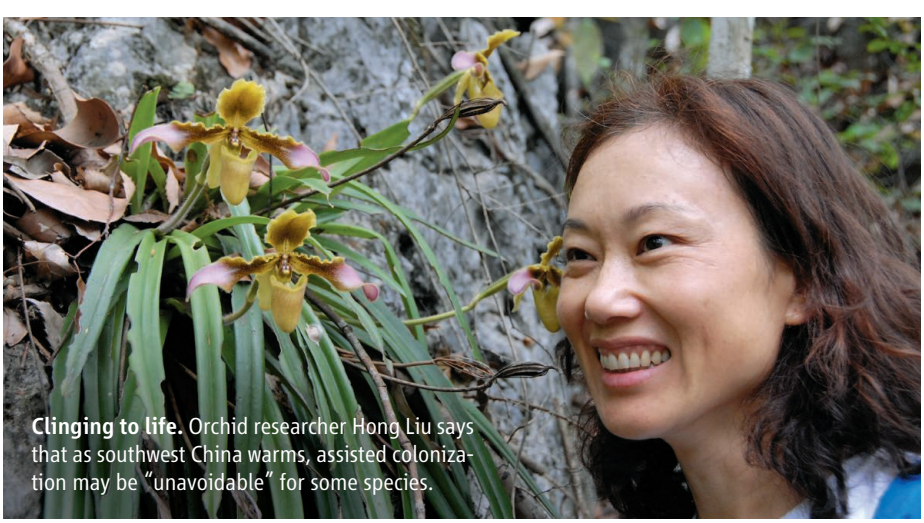
Scientists are beginning to contemplate which species might satisfy these requirements. In Australia, candidates include the greater glider and two other native possums that were victims of recent regional extirpations, says David Lindenmayer, an expert on climate-driven range changes based at Australian National University in Canberra. Another AC candidate is the Iberian desman, an amphibious insect-eating mammal whose present habitat in the Pyrenees is likely to vanish as the world warms. Since the last ice age, the Iberian desman has not expanded its range to the nearby Alps, another suitable habitat. Thus, it is unlikely to migrate on its own in the face of climate change, Naia Morueta-Holme and colleagues at Aarhus University in Rønde, Denmark, noted last April in *PLoS ONE*.

The challenge lies in assessing risk and guarding against unwelcome surprises. Transferred organisms can wipe out native species and disrupt food webs. Take, for example, the opossum shrimp. Thirty years ago, wildlife managers introduced the species into Flathead Lake in Montana to enrich the diet of kokanee salmon. It turned out that the nocturnal shrimp spent daytime at the lake bottom, while salmon fed in shallower water. So instead of becoming dinner, the shrimp competed with the salmon for food, and the kokanee crashed. A higher predator then suffered: Eagles that preyed on the salmon also crashed. "We can explain such complex effects after they have occurred," Ricciardi says, "but we can rarely predict them."

When assessing the likelihood of a species becoming an upstanding member of its new community or a vicious invader, looks can be deceptive. In the 19th century, two closely related Eurasian sparrows were introduced to North America. Since then, the tree sparrow, *Passer montanus*, has expanded its range slowly, while the house sparrow, *P. domesticus*, has spread widely and supplanted native birds. "Real knowledge of what determines the range limits of a particular species is extremely meager," says Simberloff. And ill effects of an introduced species may not be detected until years later.

In the absence of hard data, AC risk assessments boil down to guesswork. "It's really a Delphic process," says Simberloff. "Someone says the risk is high, another says it is low." Medium becomes the consensus. He is calling for "souped-up natural histories" that might be undertaken as doctoral theses about a given species. "This kind of research is not fashionable," Simberloff says, "but it's what you will need."

AC advocates acknowledge that data gaps must be filled before the approach is ready for prime time. One lacuna is how much climate variation species can tolerate beyond the conditions in their native habitats. "This has been



Clinging to life. Orchid researcher Hong Liu says that as southwest China warms, assisted colonization may be "unavoidable" for some species.

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Moving experience. In a landmark experiment, Stephen Willis and colleagues moved populations of two butterflies—the small skipper (*top right*) and the marbled white—north of their ranges. Ten years later, both species are thriving in their new homes.

worked out for a few species, but there are no general rules of thumb yet," says Dov Sax, a conservation biologist at Brown University. Another issue is the degree to which species may evolve in response to climate change. "The quick answer is that we expect species with lots of genetic variation and very short generation times to be able to adapt," Sax says. "The trouble is that we don't know where the cutoffs should be for expecting trouble."

Fundamental questions remain unanswered. As the climate changes, which creatures will migrate too slowly? And which will face insurmountable barriers? Answers may come too late for some species. But at least one study has shown that AC can work.

In what may be the first AC field trial, a decade ago Willis and colleagues moved populations of two butterfly species in England from their ranges at that time to new areas to the north. "We wanted to see whether those areas could support viable populations," says Willis. The idea was to test the feasibility of AC using a species that was not already on the ropes. There was a good shot the experiment would work. The butterflies—the marbled white and the small skipper—had pushed northward in England in recent years.

Based on species distribution models, Willis's team forecast areas of the British Isles in which temperatures and rainfall amounts in coming years should provide suitable habitat. In summer 1999, they captured 400 adult small skippers and the next day whisked them to an abandoned quarry in Northumberland 35 kilometers north of their then-range. Another 200 were moved the following July. Also, in 2000, the researchers moved 500 adult marbled whites to a reserve in Durham, 65 kilometers north of the butterfly's then-range.

The scientists have been tracking the butterflies ever since. After a rough couple of summers in 2008 and 2009, when England was unseasonably chilly, the butterflies have bounced back this year. "They are behaving very much like natural populations," says Willis. In the February 2009 issue of *Conservation Letters*, his team concluded that "assisted colonization has the potential to be a useful conservation tool" to soften the blow of climate change for species with poor mobility or whose habitat is fragmented. The study "makes a strong case that managed relocation is feasible," says Sax.





Turn on the AC? Warming threatens the Iberian desman (*left*) and the greater glider.

Dire straits

Deep in Yachang orchid reserve, a trail covered with skin-flaying bramble gives way to a sunlit meadow filled with young corn stalks. "It's almost like Iowa," mutters Liu, clearly disappointed that villagers are growing corn in the heart of orchid country. At the edge of the meadow, fresh cuts ringing the base of an oak tree are a disturbing sign. It's illegal to fell living trees in the reserve. To expand their cornfield, villagers inflict mortal wounds by girdling trees and later legally remove the dead wood. Prosecutions are rare because offenders can be fined or sent to labor camp, and that would sow enmity in a small town such as Huaping. Therefore, despite the blatant transgressions, "we have to be careful," says Yachang director Wu Tian-gui. He and his staff members don't want villagers to strike back at the reserve by poaching rare orchids that can fetch hundreds of dollars from collectors.

Meanwhile, for Yachang's orchids, the noose tightens. As the region warms, many orchids will migrate naturally, and tissue culture could augment existing populations, says Feng. But some species will be driven higher up mountainsides, and eventually "they will have no higher places to go," says Liu, who describes the threat to orchids in the June issue of *The Botanical Review*. Other orchids may be stymied as the habitat grows ever more fragmented. "Managed relocation of certain orchids may be unavoidable," she says.

It won't be easy. Orchids have a poorly understood symbiotic relationship with soil fungi. And pollinators are species-specific. New habitat for any orchid given a moving assist must have not only a tolerable temperature and precipitation regime but also the right species assemblage. AC for orchids, says Feng, "will be very tricky."

As questions swirl about how best to proceed, the plight of one species has driven people to take matters into their own hands. Blighted by disease, the Florida Torreya pine has lost more than 98% of its population since the 1950s. Over the past decade, the Torreya Guardians have been distributing seeds well beyond the tree's historic range. The private group cites climate change as one rationale for its "assisted migration."

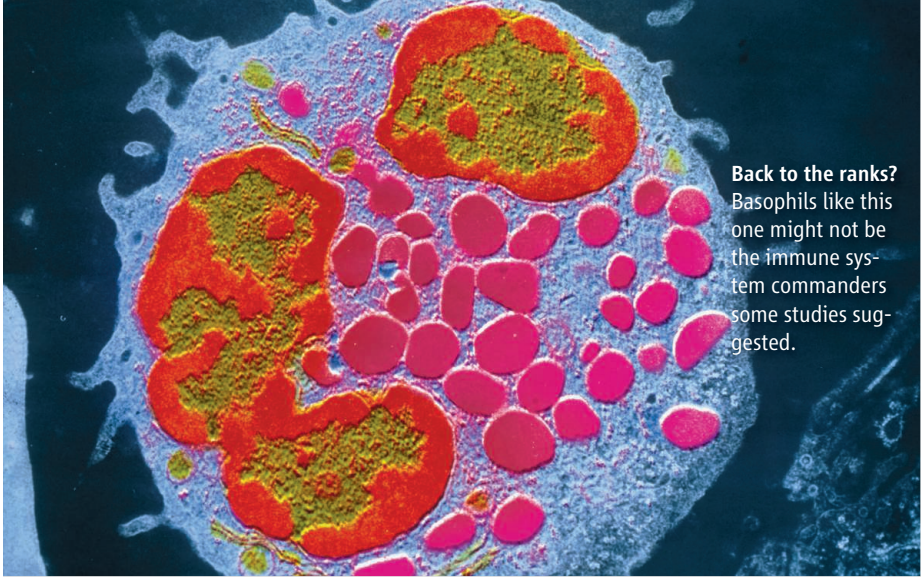
Sax sees a moral justification for this ecoactivism. "They have every right to try and fix a problem that they don't see anyone else dealing with," he says. But there is a dark side, he notes. "It makes me nervous to think that any group could move any species they wanted. This would occasionally lead to some nasty ecological consequences."

Thus, it is imperative that scientists illuminate the benefits and risks of moving species before agencies, citizens' groups, or their own colleagues take action. "Before AC can become a safe tool, we would need to develop a much better understanding of how introduced species and recipient ecosystems respond to each other," says Ricciardi. "Until then, AC amounts to ecological gambling: The more frequently we do it, the more we spin a roulette wheel of unintended consequences." **–RICHARD STONE**

CREDITS (TOP): COURTESY OF STEPHEN WILLIS; (INSETS): VALERIA GENG/WIKIPEDIA; COURTESY OF STEPHEN WILLIS; (BOTTOM) WIKIPEDIA; (INSET) DAVID PEREZ/WIKIPEDIA

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Back to the ranks? Basophils like this one might not be the immune system commanders some studies suggested.

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IMMUNOLOGY

Mouse Studies Challenge Rare Immune Cell's Powers

The functions of basophils, one of the first immune cells to be identified, continue to confound researchers

Long dismissed as "cannon fodder," as one immunologist puts it, the white blood cells known as basophils have been enjoying a renaissance. Findings published mainly within the past 5 years portrayed basophils as take-charge cells that control maturation of key defensive cells, instigate counterattacks against invaders, and even help the immune system remember. But researchers who just 2 months ago were touting basophils' powers are now using words such as "reassess" and "reconsider."

That's because three papers published this month question whether the cells perform several jobs recently ascribed to them. Some immunologists say they are already convinced that the previous basophil work was wrong, whereas other researchers declare themselves puzzled by the contradiction.

Discovered in the late 1870s, basophils remain among the most enigmatic immune cells. The consensus long held that basophils—which account for less than 1% of white blood cells—contribute little to the body's defenses. Many researchers considered them backups for mast cells, another type of immune cell that triggers allergy symptoms and defends against parasites (*Science*, 3 August 2007, p. 614).

Then about 3 years ago, researchers found they could wipe out basophils in mice by administering antibodies that grab molecules on the cells' surface. The effect was only temporary—the animals gradually make replacements—and the antibodies might also kill or activate mast cells. But immunologists still thought they finally could tease out the functions of basophils by comparing antibody-treated mice to normal animals and ones lacking solely mast cells.

One important role that emerged was guiding the maturation of "helper" T cells that orchestrate immune assaults against pathogens. These T cells can specialize to trigger a T_H1 response, which tackles viruses and certain bacteria, or a T_H2 response, which targets parasites. By producing the potent cytokine IL-4, basophils seemed able to nudge T cells down the T_H2 route. In 2008, then–graduate student Caroline Sokol, her adviser Ruslan Medzhitov of Yale University, and colleagues showed that basophils can enter lymph nodes—which many researchers had thought were off-limits to the cells—to rendezvous with T cells. They also found that removing basophils with antibodies squelched the T_H2 response.

The job attributed to basophils that has spurred the most discussion is presenting antigens. Before a helper T cell can trigger a response to a pathogen or parasite, it has to "feel" these molecular bits of an invader displayed on the surface of an antigen-presenting cell (APC). Most researchers assumed that the immune system's dendritic cells (DCs) show off antigens during T_H2 responses. Last year, however, in simultaneous *Nature Immunology* papers, three groups published evidence that basophils serve as APCs.

That conclusion is now under fire. Immunologist David Voehringer of Ludwig Maximilians University Munich in Germany, and colleagues announced in an article published online on 2 September in *Immunity* that they had genetically engineered the first mice that are almost completely devoid of basophils throughout their lives. The scientists disabled a gene crucial for the cell's development.

Even without basophils, the animals could muster T_H2 responses to allergens and parasitic hookworms. That the rodents' immune systems reacted normally to these apparent threats also suggests that basophils aren't crucial antigen presenters, the researchers conclude. "We do not believe that basophils have APC function under physiologic conditions," says Voehringer. His team did, however, find that basophils help rebuff a parasite's second attack, confirming that the cells aid immune memory.

Two new studies of mice whose basophils had been eliminated by antibodies, both appearing online in the *Journal of Experimental Medicine* this month, reach much the same conclusion: Basophils are not APCs for dust mite allergens or *Schistosoma* parasites. "It's a matter of antigen presentation efficiency," says immunologist Bart Lambrecht of the University of Ghent in Belgium, co-author of the dust mite allergen paper. In culture dish tests of APC prowess, his team found that basophils performed feebly.

Lambrecht's and Voehringer's groups note that one of the basophil-depleting antibodies can knock out certain DCs. Their demise—not the disappearance of basophils—could explain why in some studies mice lost APC capability after antibody injections. Voehringer is brusque, calling many of the earlier basophil results experimental artifacts.

The researchers who fingered basophils as APCs stand by their work. "It's hard for me to imagine that what so many groups were seeing was an artifact," says Sokol, who is now a resident at Massachusetts General Hospital in Boston. Immunologist David Artis of the University of Pennsylvania remains convinced that basophils act as APCs for some stimuli. "But are there scenarios where basophils may not participate in antigen presentation? Absolutely."

Voehringer's basophil-lacking mice don't provide definitive evidence, because other immune cells might take over basophil jobs in the animals, says immunologist Hajime Karasuyama of Tokyo Medical and Dental University Graduate School in Japan, who wasn't part of the APC research. Nevertheless, he concludes, the accumulating data suggest that "we definitely need to reassess the role of DCs and basophils." **–MITCH LESLIE**

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Consumer appeal. Fish certified as sustainable, such as these relatives of the Antarctic toothfish, can fetch higher prices.

FISHERIES

Behind the Eco-Label, a Debate Over Antarctic Toothfish

The controversial case of the Antarctic toothfish has raised questions about the gold standard for environmentally friendly fishing

In grocery stores around the world, discerning consumers can select fish that come with a distinctive blue label and a check mark. It's the most common eco-label for seafood, offering guilt-free eating in exchange for a premium price. This stamp of approval from the Marine Stewardship Council (MSC), a rapidly growing nonprofit based in London, means that the fishing operation doesn't catch so many fish that it jeopardizes the stock. Moreover, the fishing techniques should minimize collateral damage to the ecosystem, such as accidentally catching sea birds or turtles.

But how well the sticker delivers on its promise is up for debate. Critics, both academic scientists and those with environmental groups, say MSC dispenses its labels too liberally, when there are not enough data for a definitive evaluation. They also say the process of certification can be too subjective. The result, they say, is that fisheries are certified when they aren't clearly sustainable. "It's the organization that can't say no," says Gerald Leape of the Pew Environment Group in Washington, D.C.

These concerns have been reignited by a battle over the Antarctic toothfish, which lives in one of the most pristine marine ecosystems. Three fishing companies want to market it as sustainable and were on track to get the label. But environmental organizations have objected, arguing that the label isn't warranted, given the paucity of data about the life history of the toothfish and the ecological impacts of fishing in the Ross Sea. An independent examiner is now reviewing the evidence. Whatever the outcome, the saga of the Antarctic toothfish shows that making a watertight case for sustainability can be devilishly difficult. In the 2 September issue of *Nature*, several scientists called for "radical reform" of MSC.

Supporters of MSC say the process is to some degree inherently subjective; various scientists will come to different conclusions when data are scarce. They say certifiers do the best possible job, considering they have to work with imperfect data. Moreover, they account for uncertainties by imposing conditions that fisheries must meet to retain their certification—thus nudging fisheries toward even better operations. "You can eat this seafood with the assurance that it's coming from fisheries that are well-managed and the most environmentally friendly in the world," says fisheries scientist Ray Hilborn of the University of Washington, Seattle.

Making the grade

MSC was conceived in 1997 by the World Wildlife Fund and Unilever, one of the largest manufacturers of frozen fish products. Both organizations were concerned about the state of fish stocks around the world and thought that independent certification could help promote sustainable practices. After extensive consultations with scientists, MSC created a set of general standards for sustainability. A technical advisory board helps keep the assessment methods up to date.

Despite a slow start, the number of certifications has risen dramatically in recent years. Demand from grocery retailers has also increased; in 2006, Wal-Mart announced that it would sell only MSC-certified fish. Fishers prize the label, which can provide access to lucrative wholesale contracts or higher retail prices. The 94 certified fisheries produce more than 5 million metric tons a year, totaling 5% of wild-caught fish consumed worldwide.

But there were controversies from the beginning. Environmental groups challenged major decisions, such as the South Georgia Patagonian toothfish, variously pointing to the poor state of the stocks, risk of overfishing, or uncertainties about ecological impacts. None of 11 appeals to date have succeeded, although some have resulted in additional conditions being placed on fisheries.

A similar conflict is now playing out in the world's southernmost fishery. Like its Patagonian relative, the Antarctic toothfish (*Dissostichus mawsoni*) is commonly known as Chilean sea bass. Popular for their mild, fatty flesh, the slow-maturing toothfish are inherently vulnerable to overfishing. Up to 16 ships are allowed to fish for Antarctic toothfish by the Convention on the Conservation of Antarctic Marine Living Resources. Catch levels are kept low while more is learned about the species. "It's a very well-managed fishery," says Eric Barratt, managing director of Sanford Ltd., one of the three companies applying for MSC certification.

MSC sets the overarching policies and technical guidance but doesn't certify fisheries itself. That's done by a handful of research and analysis companies. Staff scientists and consultants review the scientific literature

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and assess whether a fishery's performance measures up to MSC's many standards in each of three broad areas: the status of the stock, the impact on the broader ecosystem, and how well the fishery is managed. It's a complicated grading system, and succeeding is a bit like triple majoring in college: Each major requires a minimum grade point average; and although you can't flunk any classes, if you get enough A's, then having some C's on your transcript won't hold you back. This entire report card is then peer reviewed by two scientists.

Fishing companies can select any approved certifier. Sanford and the other companies contracted with Moody Marine Ltd. in 2007 to evaluate the fishery. Because fisheries differ so much—in the life history of the species and the gear used to catch them, for example—Moody and other companies tailor the grading system case by case. They create so-called scoring guideposts, which determine what's required to get a passing score (60 points out of 100). The companies have a good deal of flexibility in how they define these guideposts, which has led to charges that certifiers tend to be too lax.





Unknown impact. Catching toothfish might deprive predators, such as Weddell seals.

Good enough?

In November 2009, Moody decided that Antarctic toothfish caught by the three companies should be certified as sustainable. A month later, the decision was appealed by the Antarctic and Southern Ocean Coalition (ASOC), an advocacy group based in Washington, D.C. After paying MSC a $23,000 fee to appeal, ASOC—backed by a group of 39 scientists—argued that the certification wasn't justified because of a dearth of key data, such as whether or how the size of the stock has changed over the past several years or the ecosystem effects of fishing there. Weddell seals are known to prey on toothfish, for example, but it's not clear how important the fish is to the seals' diet.

Moody, recognizing some of the deficiencies, had placed conditions requiring further research on its certification of the Antarctic toothfish. (Like all certifications, the fishery would be audited annually to make sure it's up to snuff.) ASOC argues that these data gaps should be filled before the fish is sold as sustainably harvested, not after.

MSC appointed Michael Lodge, a lawyer with experience in fisheries management, to check whether proper procedures were followed. In a preliminary report released in May, Lodge agreed with some of the complaints about the contested scores in the Moody assessment. Based on how little is known about the species' life history, such as reproductive behavior and larval movements, six scores that Moody had awarded the fishery were unjustified, Lodge found. He directed Moody to reconsider, but the company declined to change any of the scores.

ASOC had also complained that Moody had ignored suggestions from stakeholders and two peer reviewers. Lodge noted that where the peer reviewers had recommended lower scores, Moody kept them unchanged. Lodge described "a defensive attitude on the part of the assessment team, coupled with an unwillingness to change scores that had already been decided." Andrew Hough, a marine ecologist with Moody who led the assessment, says his company takes all comments seriously, but often peer reviewers misunderstand the report. "If we need to change the scores in light of the comments, then we do," he says.

Another issue apparent from Lodge's investigation is that various aspects of certification can be quite squishy. In setting up the scoring guideposts—that is, what's required to get a passing grade—Moody relied in many places on vague terms such as an "adequate" or a "reasonable" amount of knowledge, Lodge found. Lodge emphasized that objectively defined guideposts are crucial: "If the 60 guidepost is set too leniently, then it undermines the whole purpose of assessment." But he noted it wasn't within in his charge to rule on the adequacy of the scoring guidelines, so he let stand the scores contested on these grounds.

Subjectivity in scoring may be common, according to an analysis published in *Fish and Fisheries* in 2008. Fisheries scientist Trevor Ward, a consultant based in Perth, Australia, analyzed 22 MSC-certified fisheries and found that one major certifier systematically awarded higher scores for minimal ecosystem impact than did another.

MSC says it fixed the problem in 2008 by revising its standards for certifiers, so that there is a consistent assessment system for every fishery. For example, assessments must explicitly consider stock status in an appropriate manner. "There's a feeling among the NGO community that [the new standards] are an improvement," says Michael Hirschfield, chief scientist for the advocacy group Oceana in Washington, D.C. But Ward and others say certifiers continue to have excessive flexibility in deciding what information is adequate. What's needed, says Pew's Gerald Leape, are more absolute requirements; not just any qualitative measure of stock status, for example, but a rigorous stock assessment.

MSC and the certifiers say they only approve fisheries when the existing data support that decision. What's more, they say, the conditions required for recertification every 5 years mean that fishers big and small adopt even more sustainable fishing practices. "It's led to some very significant changes in how fisheries are managed," says David Agnew, who chairs MSC's technical advisory board.

As for the toothfish, Lodge is expected to rule on the appeal by the end of the month. Meanwhile, some 130 other fisheries are being evaluated for certification, so the toothfish most likely won't be the last controversy over the adequacy of the science. For consumers or grocery suppliers who are mulling whether to buy fish with the MSC label, the decision may boil down to whether they want to support a fishery that is incontrovertibly sustainable or just heading in that direction.

–ERIK STOKSTAD

CREDITS (TOP TO BOTTOM): JESSICA MEIR; SUNHILD WILHELMS; (INSET) MARINE STEWARDSHIP COUNCIL

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From fears of death 1601



Rejuvenated panthers 1606



LETTERS | BOOKS | POLICY FORUM | EDUCATION FORUM | PERSPECTIVES

LETTERS

edited by Jennifer Sills

Retraction

IN THE REPORT "COMBINATORIAL EFFECTS OF ODORANT MIXES IN OLFACTORY CORTEX" (*1*), WE described subcellular patterns of *Arc* (*arg3.1*) mRNA expression in anterior piriform cortex neurons after mice had been exposed to odorants. We reported that some cortical neurons express *Arc* in response to a mix of two odorants but not either odorant alone. My laboratory has been unable to reproduce this finding. I am therefore retracting the Report. I sincerely apologize for any confusion that its publication may have caused. Zhihua Zou declined to sign this Retraction.

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A Recount of Tropical Tree Species

IF ALIENS VISITED EARTH, PERHAPS THEY would wonder how many distinct life forms our planet has. With their advanced intellect and technology, they might well have the answer. It is clear from R. M. May's Perspective "Tropical arthropod species, more or less?" (2 July, p. 41) that we do not. May inadvertently adds to the confusion by indicating that New Guinea is home to roughly one-third of tropical tree species. Given that about 6000 tropical tree species are from New Guinea (*1*, *2*) out of 37,000 tree species in all rainforests (*3*), only about 16% of tropical tree species are from this region—about half the estimate provided by May.

I agree with May's conclusion that taxonomy needs more recognition, but we also need more of taxonomists' rigor, lest we confound what is already a challenging task. Without this rigor, our alien visitors might consider our science to be less dismal, as May suggests, but more careless.

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Science Standards: Averages Deceive

IN THEIR EDITORIAL "SEEKING SCIENCE STANdards" (28 May, p. 1075), A. I. Leshner, S. Malcom, and J. E. Roseman provide a flawed comparison of U.S. and foreign students. AAAS Fellow B. L. Benderly writes in *Scientific American* (*1*) that averages are not a useful metric for this comparison. Benderly summarizes work by Michael Teitelbaum, B. Lindsay Lowell, and Hal Salzman, indicating that the United States has more high-scoring students than any other country, in addition to a substantial number of the lowest-scoring students (according to the Trends in International Math and Science Study). The Editorial authors should find a more meaningful way to judge U.S. science education.

ANDREW DEPRISTO

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Science Standards: Value the Teachers

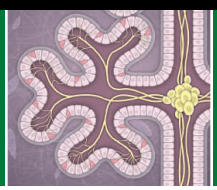
A. I. LESHNER *ET AL.*'S EDITORIAL "SEEKING science standards" (28 May, p. 1075) provides a good plan for developing consistent K–12 science standards. I would like to add that increased teacher salaries must be considered.

The United States needs to do a much bet-

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Gland growth

1610



SPORE Prize Essay

1613

ter job in attracting the best U.S. college students into K–12 teaching careers. Improved salaries will yield increased respect for K–12 teachers. South Korea, often ranked in the top tier of K–12 science achievement, has a starting teacher salary equal to 141% of the country's per capita GDP (*1*, *2*). The United States, ranked much lower, has a starting teacher salary that is only 81% of the U.S. per capita GDP (*1*, *2*). Any improvement in science education will have to include valuing teachers more highly.

The Editorial also notes that scientists must be more involved in improving U.S. science education, but this is easier said than done. Until federal research grants require recipients to be more involved in education and until U.S. universities give credit toward tenure and promotion for such activities, the culture will not change.

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Response

DEPRISTO QUESTIONS THE APPROPRIATENESS of comparing average scores of U.S. and non-U.S. students on standardized tests. Comparisons of overall scores, although not always the best metrics for what students know, can be useful indicators when attempting to understand how differences in policies, practices, resources, and attitudes lead to different outcomes (*1*).

There may indeed be adequate numbers of top-performing students who can, if they choose, contribute to the eventual base of scientists and engineers that the United States will need over the coming decades. But that misses the point. Our success will depend not only on the number of students who scored at the 90th percentile but also on the performance of the rest of the students. More than ever, all of tomorrow's workers and citizens will need familiarity and comfort with science and technology, and the bottom 10% of U.S. performers on the Trends in International Math and Science Study are among the worst performers overall on the assessment (*2*). They will be left behind in the world of the future.

Our call for addressing the needs of all students recognized that averages can mask the diversity in performance and circumstances. The story in the averages and in the percentiles is, however, instructive. The longer students are in school, the less competitive their scores become. Even the top performers lose ground. Other countries are focusing on raising the level of student achievement for both the top and lowest performers. Shouldn't we be concerned that the overall U.S. student population is lagging far behind?

We agree with Oppenheimer's point that we need to pay teachers better in order to bring the best and brightest into education. Sadly, that may be politically and, currently, economically unfeasible. Regarding bringing scientists into the classroom, many U.S. agencies (such as NSF and NASA) now require that scientists demonstrate "broader impacts" as a part of their research grants; helping precollege education to address science standards would surely meet those requirements.

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Education Framework Needs Better Foundation

I DO NOT SHARE B. ALBERTS'S ENTHUSIASM for the draft *Framework for Science Education* ("Reframing science standards," Editorial, 30 July, p. 491). For no apparent reason, the process has been insanely rushed: less than a year for a report that could have a major impact (for better or worse). The *Framework* committee met just four times before release of the draft, and then the time allotted for serious peer review was vanishingly small.

Contrary to the Editorial, I found the draft to be far from promising. Among other deficiencies, it does not support its claim to call for reduced content. It is also conceptually confusing and muddies the distinction between framework and standards. Except for its inclusion of engineering, there is little in it that is new. It vastly overemphasizes (as does the Editorial) the importance of everyone being able to "do" science and "do" engineering, and of the capacity of schools to deliver such training.

I believe that scientists and teachers would be well advised to call for a more protracted, properly peer-reviewed process, and especially one that takes time to examine thoroughly the half-century-old issue of K–12 education for understanding science versus doing science (*1*). To assume that the shortcoming of the framework will be taken care of in the next phase is to avoid our scientific and educational responsibility to get it right from the start.

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1. J. Rutherford, *J. Res. Sci. Teach.* **2**, 80 (1964).
2. The author is the former Chief Education Officer of AAAS.

CORRECTIONS AND CLARIFICATIONS

Table of Contents: (4 June, p. 1199). In the one-sentence summary for the Report by S. D. Le Picard *et al.*, OOOH was erroneously termed "hydrogen superoxide"; it should have been termed "hydrogen trioxide radical."

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 3 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

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LANGUAGE EVOLUTION

Without Social Context?

N. J. Enfield

W. Tecumseh Fitch's wide-ranging survey *The Evolution of Language* features a cautionary tale against jumping to conclusions from the anatomy of dead animals. Comparative research on mammalian vocal tract anatomy had regarded the human "two-tube" arrangement (the result of a permanently descended larynx) as unique to our species. Much was known about the anatomical structure of the vocal tract in other mammals, but nobody had looked at it in action. When Fitch (a cognitive biologist at the University of Vienna) made x-ray video recordings of goats, dogs, and deer, he found that these animals dynamically create the two-tube vocal cavity by temporarily descending the larynx, but only while actually vocalizing (*1*). This fact, which has consequences for the evaluation of competing theories of language evolution, cannot be observed when the animal is at rest (or, indeed, when dead or fossilized). The lesson is a general one: To build an adequate empirical basis for solving problems as difficult as the evolution of language, we must observe the target phenomena as directly as possible and in their proper contexts. It is therefore striking that in studying language, the dominant approaches to linguistics have pursued highly abstract analyses based on data that are only indirectly related to the phenomenon in its natural setting.

Linguists make a distinction between speech (the physical production of utterances) and language (the underlying grammar and meaning of those utterances). The great strength of Fitch's book lies in its treatment of speech. He provides a masterful ground-up survey of concepts and data in evolutionary biology that enable us to study the evolution of the vocal anatomy and its neural underpinnings. His treatment of language takes some dominant trends in linguistics to represent "over-arching areas of agreement," an interpretation that is understandable given that linguistics is not his home discipline.

The distinctly narrow conception of language foregrounded in *The Evolution of Human Language* is less justifiable, because its editors (Richard Larson, Viviane Déprez, and Hiroko Yamakido) are linguists. The chapters in this volume vary in approach but are all loosely focused on a widely discussed 2002 review article by Marc Hauser, Noam Chomsky, and Fitch, "The faculty of language: What is it, who has it, and how did it evolve?" (*2*). Each of the three coauthors contributes his own chapter following up on that paper.

In their article (reprinted in Larson *et al*.), Hauser, Chomsky, and Fitch distinguished between faculty of language in narrow and broad senses. They defined the former as an "abstract linguistic computational system … independent of the other systems with which it interacts and interfaces" and described the latter as made up of those other cognitive systems that are crucial for language but that may have broader functions. In his single-authored chapter in Larson *et al*., Chomsky maintains the centrality for language evolution of an abstract and narrow language faculty, driving a saltationist argument whose central hypothesis is that "some genetic event rewired the brain." Both Fitch (in his book) and Hauser (in his chapter) now explicitly acknowledge the possibility that the putative narrow language faculty "may be completely empty" (i.e., may not exist), and this is indeed what many researchers in the cognitive sciences currently believe. There are well-developed gradualist evolutionary arguments that language is entirely grounded in a constellation of cognitive capacities that each—taken separately—has other functions as well (*3*).

Hauser uses his chapter as a chance to defend against certain (mis)readings of his own views, while Fitch moves the debate forward by usefully distinguishing among meanings of "recursion," a concept prominent in the 2002 review and a topic of subsequent controversy. Chomsky takes his chapter as an opportunity to boldly assert his personal position on language evolution. Many linguists will feel a familiar sense of frustration at his omission or dismissal of decades of prominent and successful linguistic research that has not necessarily aligned with various influential Chomskyan programs. The problem is that nonspecialist readers will not learn from these chapters about the profound methodological and theoretical upheaval that is currently under way in linguistics thanks to the recent work of many prominent scientists of language [e.g., (*4*–*6*)]. Empirical and theoretical offerings from psychology, linguistics, and anthropology are supplying cognitive science with new approaches to language—in particular, new ways to understand the role of general cognitive capacities in making language what it is.



Communication without syntax.

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The previous focus on an abstract and narrow language faculty in linguistics has been driven by forms of research that are surprisingly far from the empirical, data-driven approach that Fitch insists is needed. The research technique long favored in generative linguistics has been to employ "grammaticality judgment" data to infer the underlying syntactic structure of sentences and, through this, to generate hypotheses about linguistic cognition. The linguist constructs imagined target sentences, checking to see if they are considered acceptable to native speakers. But such data notoriously give rise to irresolvable disagreements about whether an example sentence is in fact permissible (*7*–*9*). Not only

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are such measures unreliable, they are indirect (being metalinguistic rather than linguistic) and devoid of proper context. The conclusion to be drawn from this is analogous to that of Fitch's x-ray video studies: grammaticality judgments are the dead animals of linguistic science. Fortunately there are major lines of linguistic research that go beyond these static and circuitous methods, giving us direct access to language in its dynamic context. These approaches—hardly visible in either book—stand to be of central importance for future research on language evolution.

The Evolution of Language
by W. Tecumseh Fitch
Cambridge University Press, Cambridge, 2010. 623 pp. $120, £70. ISBN 9780521859936. Paper, $55, £29.99. ISBN 9780521677363.

The Evolution of Human Language
Biolinguistic Perspectives
Richard K. Larson, Viviane Déprez, and Hiroko Yamakido, Eds.
Cambridge University Press, Cambridge, 2010. 279 pp. $105, £60. ISBN 9780521516457. Paper, $41, £23.99. ISBN 9780521736251.

One such line of work meets Fitch's plea for data-driven research to a degree unprecedented in the history of linguistics. By exploiting newly available massive natural language corpora and powerful computing, scientists are beginning to capture the dynamics of language as a population phenomenon (*10*, *11*). Large-scale statistical analysis of variation and frequency of actual output structures from members of a speech community not only captures population-level variation in the behavioral phenotype (crucial for understanding any evolutionary process), it also quantifies the variation in input that an infant will encounter when acquiring a first language.

The ethology of language as a system for communication provides a second approach essential for understanding language evolution. Some have argued for the critical importance of language's social functions to its evolution—notably Robin Dunbar, with his "grooming and gossip" proposal (*12*), and Michael Tomasello, with his "shared intentionality" proposal (*4*). We need direct observation of the dynamics of language as a form of social behavior not just to better understand language in relation to key questions in human evolution (e.g., how language is related to our special forms of human sociality). More important, such observation offers a firm empirical handle on just what it is we are talking about when we talk about language.

For some, syntax is the sine qua non. In his chapter in Larson *et al*., Massimo Piattelli-Palmarini claims that "communication as mere speech minus syntax is not an option." But one simply needs to look at the data: normal human infants achieve rich communication without syntax when they are at the one-word stage of language acquisition (around age 12 to 18 months). Moreover, before this stage, infants can communicate without any language at all. How do they do it? By recourse to bodily forms of signaling (e.g., pointing gestures and gaze) riding on a chassis of elite human capacities for cooperation, prosociality, and naïve psychology—as stressed by Dan Sperber and Gloria Origgi in Larson *et al*. and touched on in the chapters by Derek Bickerton and Paul Bingham and in Fitch's book. We can communicate without syntax. What we can't do is communicate without a cognitive and bodily infrastructure for social interaction. But linguistics has little to say about this. The empirical domain of language in its role as a central tool for human social life remains untouched by the mainstream of linguistics and is now largely in the hands of sociologists, anthropologists, and psychologists.

As these two books show, everyone now agrees that it is possible and desirable to conduct research toward understanding the evolution of language. And everyone agrees that there is a very great deal of work to be done. An urgent prerequisite for this line of research—surprisingly not yet on the common agenda—is a comparative understanding of language (and related communicative systems) in the dynamic context of human social behavior.

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BIOETHICS

Wrong Turns from Worst Worries

Thomas May

George Annas's *Worst Case Bioethics: Death, Disaster, and Public Health* offers a valuable addition to the public policy literature. Its principal theme, how a fear of death can distort policy, should be considered by policy-makers at all levels. As readers familiar with his work might expect from the Boston University bioethicist, the book overall has a pronounced focus on human rights, which is reflected in each of its three sections.

Worst Case Bioethics
Death, Disaster, and Public Health
by George J. Annas
Oxford University Press, New York, 2010. 359 pp. $39.95, £27.50. ISBN 9780195391732.

In the first section, "Death and Disaster," Annas argues that fear of death is at the core of the challenges facing the U.S. healthcare system and that meaningful reform requires that we, as a society, confront this fear. To illustrate how such fear frames our approach, he begins with chapters examining the problems facing healthcare reform and the threats posed by bioterrorism and medical emergencies. From this basis, Annas turns to discuss how responses to our fear of death have resulted in problematic policies that threaten basic human rights; he includes individual chapters on torture and war.

The second section, "Death and the Constitution," focuses on medical decision-making concerning issues involving death in the context of constitutional judicial decisions (primarily at the level of the U.S. Supreme Court). Annas's purpose is both to provide context and to illustrate how extreme (worst case) scenarios in general, and fear of death in particular,

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warp public policy in a negative manner. Here he examines such issues as cancer, abortion, patient safety, and drug use.

In the concluding section, "Disaster and Public Health," Annas considers public health policy in the context of the fear-of-death perspective outlined in the earlier chapters. Here, the author describes the problems created by particular policies developed in the milieu of fear of death (particularly in reaction to 9/11) and the threats to human rights resulting from reactionary public policy. More important, he also offers suggestions for how public health policy could avoid these threats to human rights and in doing so be more effective. Especially interesting chapters in this section cover genetics and DNA profiling and databases.



Water from a safe source. Fears of death may divert resources from necessary social and infrastructure programs such as the provision of clean drinking water.

Throughout the book, Annas stresses an extremely important point: how the fear of death and, in the aftermath of 9/11, particularly the fear of violent or terror-related death has resulted in repressive public policy. I have argued that irrational fear has led to unsound policies to protect against bioterrorism that ignore more important needs in the public health infrastructure (*1*, *2*). Along these lines, the chapter "Statue of Security" offers an excellent description of how fear has misdirected our focus to interventions that are at best ill-advised and at worst irrational (my terminology).

At a broader level, however, perhaps the most important chapter is "Global Health." Although he takes up the issue of globalization (including poorly regulated clinical trials in Africa, Asia, Eastern Europe, and Latin America) at the end of the chapter, Annas concentrates on the relationship of justice, human rights, and inequalities that exist among countries. At the outset of the chapter, he discusses epidemic disease (and especially AIDS) as a challenge that will require population-based prevention grounded in a recognition of human rights. Nonetheless, we might argue about Annas's own focus here at a deeper level: that foreign health aid policy itself—and in particular its emphasis within global infectious disease prevention—reflects a distortion framed by our own society's fear of death, similar to what Annas argues for throughout his book. To see this, let us consider the core of foreign health aid (and specifically aid to check the spread of infectious diseases) and its relationship to more basic health and economic inequalities.

Infectious disease constitutes one of the gravest problems facing developing countries (that it no longer does so in industrialized nations may account for the lack of appreciation for the value of public health infrastructure that has characterized the United States in recent decades). Whereas in economically developed countries only 8% of mortality is attributable to communicable diseases, elsewhere such illnesses are responsible for 56% of mortality (*3*) and account for up to three times the morbidity and mortality caused by heart disease, cancer, and strokes combined (*4*). Without sound public health infrastructure, this burden is likely to grow: Continuing migration to cities, which brings people into ever-closer contact, is increasing the susceptibility of developing countries to infectious disease (*5*). In 1950, roughly 18% of the population of the developing world was urban; by 2000, the portion had jumped to 40% (*6*). The capability to monitor and contain infectious diseases is of fundamental importance for improving population-wide health under such circumstances. Yet, one might argue, the developed world's approach is largely framed by obsessions and fears of death.

For example, consider the substantial emphasis that international health aid has placed on AIDS. It is (correctly) thought not only that addressing this disease will improve health but also that checking this scourge will result in economic benefits. Nonetheless, because of the high costs associated with treatment and the limited assets available to developing countries, infectious diseases like AIDS divert governments' attention and resources from other necessary infrastructure and social programs, such as sanitation, the provision of clean water, and education. According to a 1999 report of the World Health Organization, for example, in sub-Saharan Africa, the cost of treating one AIDS patient for one year equals that of educating 10 primary school students for the same amount of time (*7*). It remains an open question whether AIDS in particular should be prioritized so highly within foreign health aid programs or whether this priority largely reflects fears felt by the donors.

Although most economic analyses of the costs of infectious disease in the developing world focus on AIDS, malaria, tuberculosis, leprosy, river blindness, and other transmissible illnesses generate substantial direct and indirect economic burdens as well (*8*). The lack of public health infrastructure capable of early detection of disease outbreaks can escalate these expenses. For example, Lyme disease, which the U.S. Centers for Disease Control and Prevention estimates can be remedied for less than $80 in direct medical costs when diagnosed early, can exert a toll of $2000 to $6500 or more per patient if not detected and treated in a timely manner. Such costs are considerable, and they account for a staggering percentage of government expenditures in many developing countries—e.g., in Burkina Faso, Chad, the Congo, and Rwanda, the costs of malaria alone are estimated at 1% of the regional gross domestic product (*8*). Thus, it seems the case that in many places, an emphasis on establishing at least an effective basic public health infrastructure would pay greater dividends than a more narrow focus on AIDS.

In short, *Worst Case Bioethics* offers a valuable consideration of how public health policy is sometimes shaped by fear in a counterproductive manner. The book is well written, well reasoned, and persuasive. I recommend it highly to all who are interested in bioethics, public health policy, or national security policy.

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GLOBAL DEVELOPMENT

Family Planning and the Millennium Development Goals

To alleviate global poverty, investments and partnerships in family planning are needed.

Willard Cates Jr.,[1] Quarraisha Abdool Karim,[2,3] Wafaa El-Sadr,[3] Debra W. Haffner,[4] Gladys Kalema-Zikusoka,[5] Khama Rogo,[6] Tricia Petruney,[1] E. Megan Davidson Averill[1]*

The United Nations' (UN's) eight Millennium Development Goals (MDGs) (*1*) are widely accepted as the primary path to alleviating poverty worldwide. This month, world leaders convene to assess progress toward these goals (*2*). In the countdown to the MDG 2015 deadline and amid protracted economic recession, we need the most efficient, effective, and evidence-based means to accelerate progress toward all MDGs. Challenges must be considered in concert, and solutions must provide multidimensional dividends for the world's poor, or we risk unwisely dividing limited resources and diluting their impact. As authors from diverse communities, we emphasize here the influence that investments in rights-based family planning can have on achieving the MDGs (for endorsements, see supporting online material).

Over 215 million women who desire safe, effective family planning lack access (*3*). Population momentum (*4*) has created ever larger reproductive-age cohorts. Although advocacy for international family planning has recently intensified (*5*–*7*), in the decade since the MDGs' launch, real dollars invested in family planning have fallen (*8*). Although some gains have been made, improvements to child (MDG 4) and maternal health (MDG 5) lag far behind 2015 targets. Promoting women's reproductive rights and improving access to voluntary contraception reduces neonatal and maternal morbidity and mortality, including that attributable to unsafe abortions (*9*).

Family planning is also a cost-effective, cross-cutting intervention for achieving MDGs 1 to 3 and 6 to 8 (*10*). Specifically, family planning promotes the following:

Development (MDG 1). Robust family planning results in healthier, more economically stable families, communities, and nations. Each dollar spent on family planning can save up to $31 in health-care, water, education, housing, and other costs (*11*).

Education (MDG 2) and empowerment for women and girls (MDG 3). Given control over their fertility, girls are more likely to stay in school (*12*) and women to be employed (*3*).

HIV prevention (MDG 6). Family planning provides dual protection against unintended pregnancy and HIV transmission. This helps reduce mother-to-child HIV transmission (*13*), decreases the number of children born with HIV infection, and reduces obstetrical complications (*14*).

Environmental conservation (MDG 7). Family planning is a cost-effective way to preserve environmental resources (*15*). Yet women in regions facing challenges to environmental sustainability have limited access to family-planning resources. Poor families in these areas must often resort to unsustainable agricultural practices to survive, which can increase the spread of infectious zoonoses and threaten vulnerable habitats (*16*, *17*).

Partnerships across diverse ideologies (MDG 8). An expanding array of stakeholders supports family-planning principles, including faith-based institutions committed to improving women's and children's lives by championing global reproductive health. Many religious and spiritual leaders affirm that the sacredness of life is best upheld when individuals and families can create life intentionally in environments where children thrive and women's welfare is protected (*18*).

We call upon global leaders and UN MDG Advocates (*19*) to ensure better access to reproductive health for all. Leaders from diverse disciplines should acknowledge and promote family planning's multiple benefits for global development and health. UN agencies should build on *The Joint Action Plan for Women's and Children's Health* to reinforce existing and secure new commitments, and establish an accountability framework for delivering results (*20*). Member parties should honor commitments to fully finance the International Conference on Population and Development Program of Action (*21*), meet official development-assistance obligations outlined in the Monterrey Consensus (*22*), and execute the Abuja Declaration (*23*). By expanding discourse on development solutions, establishing nontraditional partnerships, and increasing resources for reproductive health and family planning, we can fulfill our MDG promises to the world's poor.

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24. Support provided by the William and Flora Hewlett Foundation. Special thanks to G. West, G. Raley, and S. Morrison for guidance.

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ASTRONOMY

More Supernova Surprises

Spectroscopic observations of the supernova SN1987A are providing a new window into high-energy shock physics.

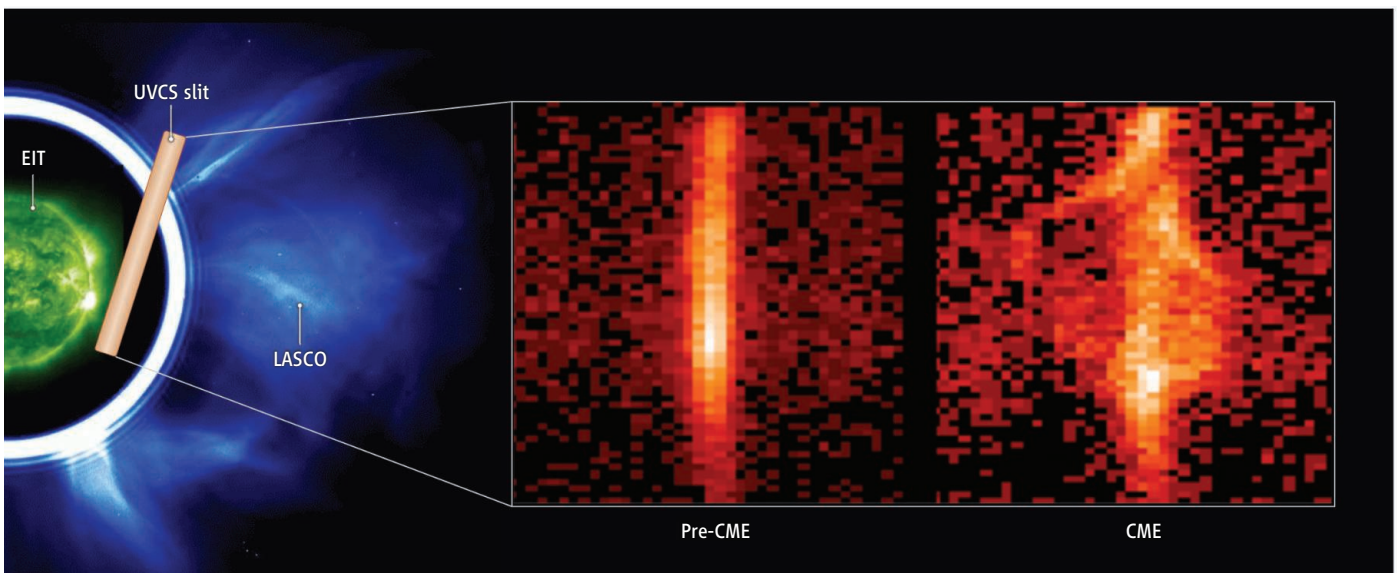
J. Martin Laming

The explosions produced when the central region of a massive star ceases nuclear burning and collapses to a neutron star (a core-collapse supernova) have stretched and motivated research that has expanded our knowledge of astrophysics. The brightest such event in modern times was the explosion of the supernova SN1987A on 23 February 1987. As SN1987A evolves from supernova to supernova remnant (SNR) and interacts with its external medium, it continues to reveal new insights into unexplored areas of physics. On page 1624 of this issue, France *et al*. (*1*) discuss how ultraviolet spectroscopy can provide new insights into the nature of shock waves associated with SN1987A.

The explosion of a supernova sends a forward shock out into the surrounding medium, driven by the motion of the explosion products (the ejecta). As the forward shock sweeps up more material, it decelerates. This deceleration must be communicated to the expanding ejecta, and in supernova remnants this usually occurs through a second backward-pointing (or reverse) shock (*2*, *3*). Spectroscopic emission line intensities and profiles (through the distribution of Doppler shifts of emitting atoms or ions) tell us important things about the condition of the explosion products and their interaction with the reverse shock. Observations with the Space Telescope Imaging Spectrograph onboard the Hubble Space Telescope (HST-STIS) reveal UV lines from neutral hydrogen and quadruply ionized nitrogen, with the distinct possibility of several more line detections with the new Cosmic Origins Spectrograph (HST-COS).

A crucial property of the shock is that it is "collisionless" (*4*). In hydrodynamics, a shock is a discontinuity in fluid properties, occurring over an infinitesimally small distance. Simple considerations of the kinematic viscosity suggest that this transition should occur over a distance similar to the collisional mean free path of a fluid particle (*5*, *6*). However, in the dilute plasmas encountered in astrophysics, the mean free path of shocked fluid particles in many SNRs is typically larger than the size of the object itself. The shock transition must occur on a length scale much smaller than the collisional mean free path. This can occur as the plasma particles interact not through the emission and absorption of virtual photons, as would be the case in vacuum, but through the emission and absorption of the quanta of oscillating electric and magnetic fields associated with collective motions of the plasma. In low-energy (or nonrelativistic) shocks, these quanta are usually Alfvén waves (low-frequency traveling waves of oscillating ions and magnetic field) and can be generated by stimulated Cerenkov radiation emitted in instabilities. Thus, if such a mode is allowed to grow by the plasma conditions, it can overwhelm the more usual Coulomb collisions (between charged particles). Hence, thermal equilibrium is no longer enforced, and phenomena associated with collisionless shocks such as particle acceleration may occur.

Neutral atoms, such as hydrogen, do not "see" these collective oscillations and penetrate right through the shock, emitting photons as they are excited by collisions with gas particles on the longer length scale. Thus, their spectral line profiles are determined by Doppler shifts associated with their preshock velocity distribution. Quadruply ionized nitrogen, along with other ions expected to be detected with HST-COS because they are electrically charged, will react to these collective oscillations, and their line profiles (also excited by collisions) will reveal information about the scattering processes that form the shock.



Shocking images. Observation of the 21 April 2002 CME by instruments onboard SOHO, with speed of 2400 km s^{-1}. The left panel gives context images: the solar disk observed in the light of Fe XII (11 times ionized, Fe^{11+}) by the Extreme-Ultraviolet Imaging Telescope (EIT) and a white-light image of the CME taken by the Large Angle Spectroscopic Coronagraph (LASCO), with the position of the UVCS slit shown. The center and right panels show "before" and "after" images of the O-VI (quintuply ionized oxygen) spectral line profile as the CME goes by, detected by the UVCS instrument. The horizontal dimension is the spectrometer dispersion direction; the vertical dimension gives spatial imaging.

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Similar ground is being broken in studies of shocks in the solar system (*7*, *8*), where phenomena related to particle acceleration have more than just academic interest. The figure shows a spectroscopic observation by the Ultraviolet Coronagraph Spectrometer (UVCS) instrument onboard the ESA/NASA Solar and Heliospheric Observatory (SOHO) of a 2400 km s^{-1} disturbance driven by a solar coronal mass ejection (CME) based on images published in (*9*). The line profile in the post-CME gas (right panel) is broader than in the pre-CME image (center panel), possibly implying through the Doppler shifts that the post-CME gas is hotter. However, the broadening is distinctly nonuniform in the spatial direction, for reasons that are currently not completely understood, but which may relate to the upstream medium or whether the disturbance has fully transitioned to a shock. To date, most work in this area has concentrated on in situ observations of planetary bow shocks, although some higher-speed shocks driven by CMEs have also been observed. The nonlocality of the collisionless shock problem often stymies quantitative understanding, as the whole shock is not observed at once; only shock parameters along the spacecraft line of flight can be detected. The four spacecraft of ESA's Cluster mission go some way toward ameliorating this, but, as can be seen in the figure, four in situ observation points instead of one do not necessarily solve the whole shock problem.

We can anticipate similar data when HST-COS observes the somewhat faster shocks in SN1987A. One might consequently expect the broadening to be even more dramatic. We are doubly fortunate in that SN1987A not only exploded at a time when ideas about the core-collapse explosion mechanism were beginning to reach a degree of maturity that could really be informed by observations, but did so in a part of the sky accessible to ultraviolet observations, thereby allowing us to study the shock waves associated with its remnant in detail.

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BOTANY

The Rise of Sunflowers

A new fossil suggests the family that includes sunflowers and daisies originated in South America.

Tod Stuessy

Everyone appreciates the beauty of daisies, chrysanthemums, and sunflowers, and many of us enjoy eating lettuce and artichokes. These cultivated plants, along with 23,000 other wild species, make up the sunflower family, also known as Compositae or Asteraceae. Today, members of the family are found on every continent except Antarctica, especially in temperate or higher elevation areas in the tropics. Where the sunflower family first evolved and how it spread, however, is not well understood, in part because researchers have found relatively few fossils. On page 1621 of this issue, Barreda *et al*. (*1*) describe an unusually well-preserved new fossil that sheds light on the history of this successful plant family and adds to evidence that it originated in southern South America about 50 million years ago.

The family Compositae is a distinct group characterized by the combination of several morphological features. The most conspicuous is flowers that are closely aggregated into heads (an arrangement that botanists call an inflorescence). The well-known sunflower has two types of flowers in the head, disc and ray flowers, but the common dandelion and many other species contain only one. In all cases, the flowers are surrounded by leaflike structures called involucral bracts or phyllaries. Another important feature is the pappus, which is a modified calyx attached to the apex of the ovary. In dandelions, the pappus extends into a parasol as fruits mature, which aids dispersal. Laterally fused pollen sacs (anthers) that form a tube, through which the female style pushes upward and presents pollen, is also a diagnostic feature for Compositae.

Despite its enormous size, broad geographic distribution and distinct features, there is a weak fossil record for this group. Most reports have been of fossil pollen (*2*, *3*), from Africa, Australia, and South America. Although variations in pollen grains exist in the family, a lack of broad diversity limits researchers' ability to determine relationships and derive evolutionary insights. The paucity of larger macrofossils has also been a problem. There are some macrofossils from the very recent Pleistocene (~2.6 million to 12,000 years ago). An older fossil, *Viguiera cronquistii*, came from western North America and is dated to the Oligocene-Miocene (~34 million to 5.3 million years ago) (*4*). Critical investigations (*5*), however, have cast doubt on the fossil plant's relationship to Compositae; it may not even be a flowering plant.



Roots. The sunflower and other members of its large family appear to have evolved from a common ancestor living in southern South America more than 50 million years ago.

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Researchers have had more success in developing hypotheses about Compositae's evolutionary sequence (phylogeny) by using molecular techniques to study genetic similarities among existing species. These studies initially identified two related families: Goodeniaceae, centered in Australia; and Calyceraceae, which are in southern South America and are closer to Compositae (*6*, *7*). Calyceraceae, a small family of just four genera and about 60 species (*8*), stands in contrast to the huge sunflower assemblage of 1500 genera. The phylogenetic approach has helped researchers identify what appears to be the oldest known familial relative: Barnadesiinae, a little known subtribe of Mutisieae; it consists of nine genera and 91 species from southern and Andean South America (*9*, *10*).

All data suggested that the common ancestor of Goodeniaceae, Calyceraceae, and Compositae developed in Antarctica, when it had mixed temperate and tropical forests (*11*, *12*). As Antarctica cooled during Eocene-Oligocene (~56 million to 23 million years ago) (*13*), the ancestral form dispersed and migrated eastward into Australia, resulting eventually in Goodeniaceae, and westward into southern South America, leading to the progenitor of Calyceraceae and Compositae. The splitting of Calyceraceae and Compositae, therefore, would have occurred in southern South America during the Eocene (~56 million to 34 million years ago). These hypotheses have been buttressed by "molecular clock" studies, which suggest that Compositae diverged in the Eocene, approximately 50 million years ago (*14*).

Given this background, Barreda *et al*.'s report of an Eocene fossil from southern South America showing clear flowering heads with phyllaries and pappus is important. At long last, there is clear macrofossil evidence of the sunflower family at an early stage of its diversification, just where it had been hypothesized to originate. The fossil does not allow unequivocal assignment, but the authors suggest that its large, conical heads and types of pappus and phyllaries are broadly compatible with Mutisieae. There is also dispersed pollen found in the matrix with the fossil, and its features are also suggestive of Mutisieae (or possibly Carduoideae). Detailed scanning and transmission electron microscopic studies on the pollen would be helpful for deeper understanding of relationships, as would finding pollen in situ in anther sacs of a better preserved fossil.

Much remains to be learned about the evolution and biogeography of the sunflower family. A new book (*15*) has synthesized molecular phylogenetic studies and, in consort with the new fossil reported here, provides strong stimuli for further research. Even if researchers accept the sunflower's origin in southern South America, it is still unclear how the family quickly colonized the entire planet and became so incredibly diverse.

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GENETICS

A Bit of Texas in Florida

Florida's inbred panthers benefited from the import of Texas pumas.

Craig Packer

Harassed, hunted, and restricted to ever smaller areas, most populations of large carnivores are fragmented into archipelagoes of parks and reserves. Biologists have long warned of the negative genetic consequences of inbreeding in such small populations. To restore genetic health, they have prescribed "active management," including moving, or translocating, individuals into inbred populations. In a time of budget cuts and inadequate funding for effective conservation, however, is translocation worth the costs? Moving a lion from Namibia to South Africa is not a trivial exercise, nor is the translocation of cougars from one part of the United States to another. But it may be worth the trouble, Johnson *et al*. (*1*) report on page 1641 of this issue. In the most comprehensive study ever conducted on the effects of inbreeding in wild carnivores, they find convincing evidence that the "quality" of a population of Florida panthers was successfully improved by the addition of panthers from Texas.

Florida panthers (also called cougars, pumas, or mountain lions) have been studied in considerable detail since the 1970s and provide an exceptionally clear example of the genetic consequences of prolonged inbreeding. By the early 1990s, Florida's population of 20 to 25 adult panthers was showing lower genetic variation than other puma populations. Biologists observed a range of problems—including heart defects, poor sperm quality, poor fecundity, and many adult males with one or no descended testes—that led to predictions that the population could go extinct within decades. In a bid to stem the tide, managers introduced eight female Texas cougars to Florida in 1995.

By comparing genetic data collected from 591 Florida panthers between 1978 and 2009, Johnson *et al*. show that Texas-Florida hybrid offspring have replaced the original inbred stock. The researchers documented increased levels of genetic heterozygosity (having different versions of the same gene), and the hybrid offspring enjoyed greater viability and fewer genetic abnormalities. The adult hybrids were also superior competitors: The pure-bred Florida panthers suffered greater mortality from fights with outbred cougars, and hybrids were better able to climb trees when pursued by scientists.

The size of the panther population has also increased since the translocation, but this result is more difficult to interpret. The amount of land available to the Florida panther has increased in the past few decades due

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Gene flow. The Florida panther got a genetic boost from introductions of pumas from Texas, but other big cats still face serious problems around the world.

to a variety of conservation measures. More land supports more cougars, so it is hard to estimate how much of the population growth resulted from the influx of "fresh blood" as opposed to range expansion. A similar translocation of 16 lions into a highly inbred population in Hluhluwe iMfolozi Park, a fenced reserve in South Africa, also improved the reproductive performance of the lions in the park, but population size did not increase in the short term (*2*).

Although translocation looks to be an effective technique for ameliorating the genetic consequences of small population size, the larger problem still remains. Big cats may be popular in places where they've become scarce and most people live in cities, but the rest of the world still struggles to deal with the dangers that man-eaters and cattle-killers pose to rural residents. Lions attacked more than 100 Tanzanians every year for the first few years of this millennium (*3*), and thousands of livestock are killed by lions, leopards, and jaguars throughout the world each year.

Retaliatory poisoning is increasingly common in Africa, greatly reducing the number of large carnivores outside national parks, and trophy hunting is excessive and poorly regulated, resulting in rapid population declines in many jurisdictions (*4*). Even in the United States, attitudes toward cougars vary from state to state. Montana paid bounties for dead cougars between 1908 and 1911; the take averaged about 140 animals per year. In contrast, between 1997 and 1999, trophy hunters in Montana killed an average of 800 cougars per year—virtually at the same time as the translocation from Texas to Florida. In 2006, Oregon announced plans to increase trophy hunting in order to decrease the state's cougar population by 40% and thereby reduce livestock depredation.

Although 21st-century Floridians may be willing to enlarge panther habitat, the story is still quite different in the rest of the world. We can perhaps take some consolation from Johnson *et al.*'s study: Once the entire planet reaches the same state of economic development and urbanization as the United States, wildlife managers all over the world can look forward to carting rare species from one park to another until the end of time.

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GENETICS

Exposing a DUX Tale

A DNA sequence stabilizes the expression of a gene that may affect muscle development and lead to muscular dystrophy.

Mani S. Mahadevan

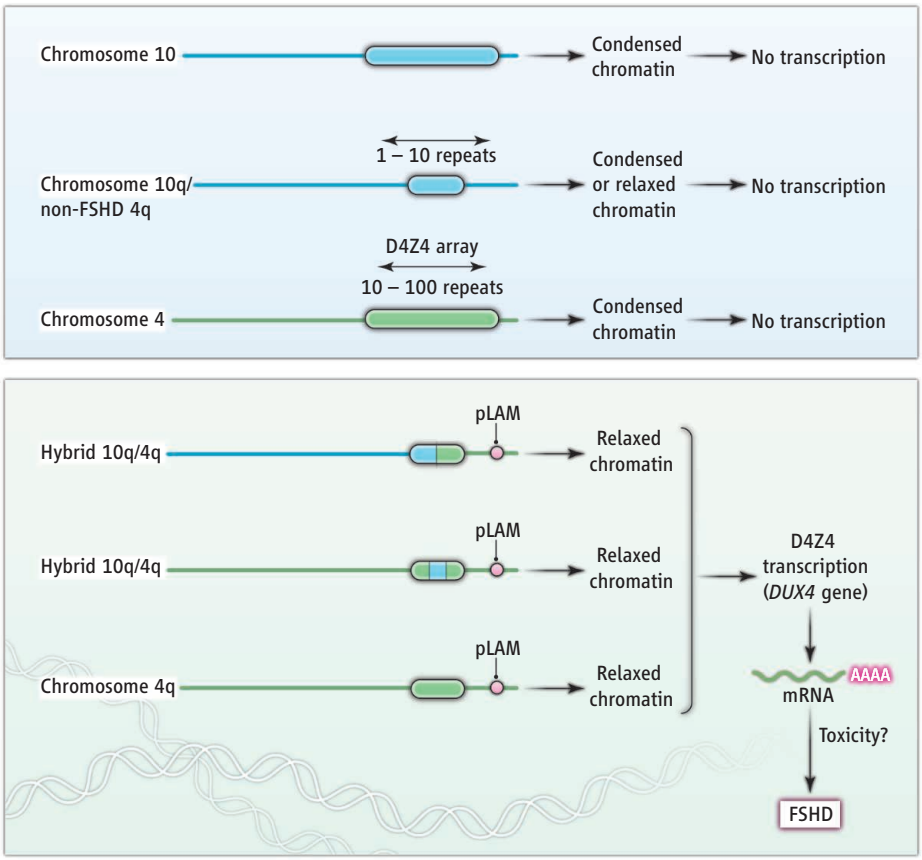
Facioscapulohumeral muscular dystrophy (FSHD), the third most common muscular dystrophy, is characterized by progressive weakness that starts in the facial muscles, proceeds to the upper back (scapula) and shoulder-upper arm regions (humeral), and eventually affects the trunk and lower extremities. Since 1992, this disorder has been associated with an array of repeated DNA sequences (called D4Z4) on chromosome 4 (*1*). An unaffected chromosome 4 has between 11 and more than 100 repeat units within D4Z4, but when this is shortened to 1 to 10 units, disease develops (see the figure). How this contraction leads to disease has been a mystery. Over the past 3 years, analyses of chromosome 4q35 have identified a combination of DNA sequences (haplotype 4A161) associated with susceptibility to FSHD, suggesting that specific sequence variations are coupled to disease pathogenesis in conjunction with D4Z4 contraction (*2*). On page 1650 of this issue, Lemmers *et al.* (*3*) provide an intriguing unifying model for FSHD pathogenesis based on very high resolution haplotype mapping and sequence analyses and careful study of exceptional pedigrees.

FSHD pathogenesis has been one of the most puzzling enigmas in human genetics for the past two decades, but there was always a consensus that the disease was caused by a gain-of-function mutation (*1*). Each D4Z4 repeat unit has a sequence called *DUX4* that potentially encodes a double homeobox gene putatively involved in developmental regu-

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Polyadenylation polymorphism. An intact D4Z4 region in chromosome 4 leaves a condensed chromatin state that prevents transcription of the distal *DUX4* gene. Chromosome 10q (and non-FSHD chromosome 4 alleles) does not have functional *DUX4* transcription elements. In FSHD, D4Z4 contractions in chromosome 4 (4q) lead to an open chromatin that exposes the distal *DUX4* gene and a sequence (pLAM) that encodes a poly(A) signal. This results in the transcription of *DUX4,* which may be linked to muscle degeneration in FSHD.

lation. Initially, the lack of clear evidence that *DUX4* is transcribed into RNA turned the focus of hypotheses toward the derepressive effects of D4Z4 contractions on gene expression through chromatin remodeling, either in cis (on neighboring genes in chromosome 4) or in trans (global effects on gene expression). But genetic analyses have brought the primary causal role of D4Z4 contractions into question, because a highly homologous region of repeats on chromosome 10 (10q) is often subject to substantial deletions without effect. More importantly, contractions to between 1 and 10 copies of the repeat array on chromosome 4q35 have been found in unaffected individuals.

Lemmers *et al*. have identified a potential polyadenylation [poly (A)] signal encoded in a region called pLAM, just distal to the last D4Z4 unit, in haplotype 4A161. This signal is not present on contracted D4Z4 arrays of non-FSHD alleles, and also not present on the shortened arrays of chromosome 10q. This is due either to the absence of the pLAM region or to single-nucleotide differences within the poly(A) signal sequence. Poly(A) signals [most commonly, the nucleic acid sequence AATAAA or ATTAAA (A, adenine; T, thymine)] enable the addition of poly(A) tails to messenger RNA (mRNA) transcripts. The absence of a poly(A) tail leads to instability and rapid degradation of mRNAs. However, the *DUX4* genes lack a poly(A) signal. Lemmers *et al*. reasoned that the poly(A) signal in the pLAM region could perhaps provide stability to a *DUX4* transcript from the distal D4Z4 unit. Indeed, *DUX4* transcripts were expressed only from "permissive" chromosome 4q (4A161) alleles. Point mutations that converted the sequence encoding the poly(A) signal to the corresponding sequences of the "nonpermissive" chromosome 10q haplotypes rendered the 4A161 alleles ineffective in expressing *DUX4* mRNA. Conversely, chromosome 10q alleles expressed *DUX4* mRNA when the 4A161 poly(A) signal sequence was swapped in for 10q sequences.

Notably, by studying FSHD patients with unusual hybrid arrays containing a mix of D4Z4 repeats from both chromosome 4q and chromosome 10q, Lemmers *et al*. show that the source of the D4Z4 units (either from 4q or 10q) is not important; it is the presence of a terminal D4Z4 unit in chromosome 4q along with the flanking pLAM sequence that accounts for disease. This was also true for a FSHD patient with a sequence variation that mapped to the chromosome 10q D4Z4 region, thus discounting a role for proximal chromosome 4q genes in FSHD pathogenesis. In cell-based assays, these hybrid arrays expressed polyadenylated *DUX4* mRNA. Lemmers *et al*. also show that *DUX4* mRNA is expressed only in muscle from FSHD patients and not from normal individuals.

Recently, the distal D4Z4 unit was shown to be sufficient for *DUX4* transcription (*4*), and there is accumulating evidence for *DUX4* expression in FSHD and its deleterious effects on muscle differentiation and development (*5*–*7*). Lemmers *et al*. now provide a molecular mechanism for the link between FSHD and chromosome 4q (4A161), resolving many of the conflicting hypotheses that have arisen due to the complex nature of the D4Z4 contractions and associated gene rearrangements between chromosomes 4q35 and 10q.

The *DUX4* gene is present across many species, suggesting an important evolutionarily conserved function, most likely in development (*8*). Many questions remain about its function and regulation, and the role of the D4Z4 array in this regulation. Contractions of the D4Z4 array are associated with more open chromatin architecture, presumably enabling transcription of *DUX4* from the distal D4Z4 unit. It would be interesting to assess *DUX4* expression in patients with FSHD2, a variant form of FSHD that is not associated with D4Z4 contractions, yet also has this open chromatin state. The mechanism through which this occurs is unclear (*9*). Nevertheless, as *DUX4* emerges as a potential lead therapeutic target, the stage is set for further advances in understanding FSHD pathology.

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PHYSICS

STM Ready for the Time Domain

The relaxation of a spin on a surface was imaged with a scanning tunneling microscope at 50-ns intervals.

Markus Morgenstern

The capabilities of the scanning tunneling microscope (STM) have moved well beyond locating atoms on surfaces. The STM has been used to image electronic wave functions (*1*) and magnetic structures (*2*). It has also emerged as a tool to measure the energy of the vibrations of adsorbed molecules (*3*) or surface phonons (*4*), as well as spin excitations of adsorbed species (*5*) or surface spin waves (magnons) (*6*), by using so-called inelastic spectroscopy. One way to follow how excited states evolve is to create them with an electric field or a laser, and then track their decay with a spectroscopic probe. On page 1628 of this issue, Loth *et al.* (*7*) show that the tunneling electrons from an STM tip can be used to pump electron-spin excitations. They followed the dynamics of relaxation of this electron spin with atomic-resolution STM imaging that used tunneling electrons with lower energy (see the figure). Their experiment opens up studies of the nanosecond dynamics of individual nanostructures, which has been a goal for surface scientists since the invention of the STM. Time-resolved STM experiments have been performed previously but have lacked spatial resolution (*8*, *9*).

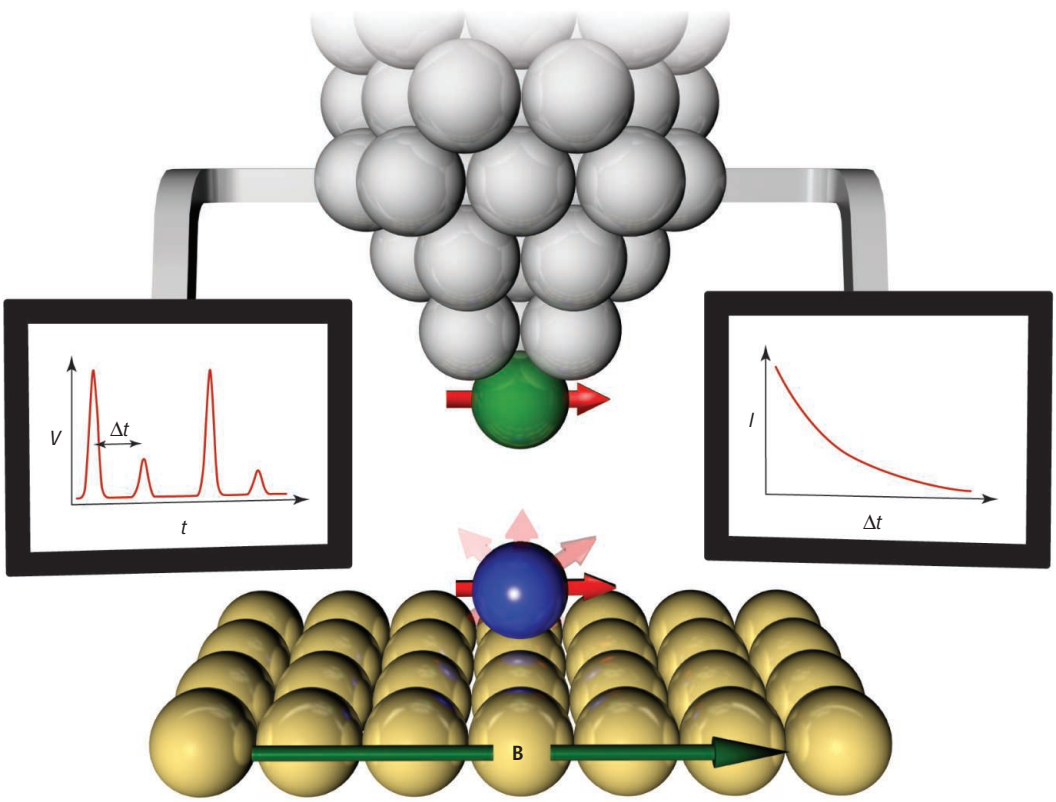
Loth *et al.* studied the electron-spin dynamics of an atomic iron-copper (Fe-Cu) dimer. They used the STM tip to assemble this dimer on a thin, insulating, copper-nitride layer that had been grown on top of a copper surface. The Cu atom helps stabilize the Fe atom, which has a spin state $S = 2$. The presence of the insulating layer increases the energy required to flip the spin, i.e., it provides a large magnetocrystalline anisotropy.

In the presence of an external magnetic field, spins will align with the field. Excitation by tunneling electrons from the STM tip provides the "pump" that flips the spin of the Fe atom antiparallel to the field. Loth *et al.* applied a short pulse (50 to 100 ns) with a high enough voltage (on the order of tens of millivolts) to overcome the energy barrier caused by the magnetocrystalline anisotropy and flip the spin. Although this excitation process does not flip the spin every time, it statistically increases the population of the excited state because it transfers energy from the tunneling electron to the spin of the dimer.

After a time delay, the excited-state spin population is probed by a second voltage pulse with a voltage lower than the excitation energy. Using a simple trick, Loth *et al.* measured the remaining population of the excited state. They decorated the STM tip with a single magnetic manganese (Mn) atom, and its spin state favors tunneling into the parallel spin, which is the ground spin state of the dimer. Thus, the tunneling current in response to the second voltage pulse increases with decreasing population of the excited state.

The temporal development of the excitation back toward its equilibrium ground state was determined by varying the time delay between the two voltage pulses. The relaxation of a single spin cannot be tracked, because this would require a forbidden, continuous measurement of a quantum-mechanical entity. However, the statistical properties of the relaxation process were determined by repeating the pulse sequence about 10^5 times for each time delay.

Loth *et al.* found that the relaxation of the spin decays exponentially with a time constant on the order of 100 ns. The magnetic-field dependence of the decay time showed that the



Following a spin's back-flip. In this schematic representation of the experiment of Loth *et al.*, a magnetic field **B** causes the ground state of the spin on an adsorbed Fe atom (shown in blue) to point to the right. A voltage pulse from the STM tip above it initially causes a spin excitation by the tunneling electrons—the spin flips against the magnetic field to the left. Subsequent smaller voltage pulses (upper left) applied after a time delay Δt trace the exponential relaxation of the tunneling current I for the spin (upper right). The evolution of the ground spin state can be followed because a magnetic Mn atom (green) on the tip favors tunneling from this orientation. The relaxation curve is statistical in nature and is built up from hundreds of thousands of relaxation experiments for each time delay.

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main pathway for spin relaxation is quantum tunneling between the two opposite directions of the $S = 2$ spin. Quantum tunneling allows the spin to shortcut the energy barrier created by the magnetocrystalline anisotropy. Thermally excited pathways would be inefficient at the low temperatures used (0.6 K).

One reason why this experiment is so exciting it that electrical pump-probe cycles are routinely used to study the dynamics of many types of spin-excitation processes, and these can now be studied with unprecedented spatial resolution. Pump-probe cycles are typically used, e.g., to observe extremely fast switching of magnetic elements (*10*) and to coherently manipulate and track spins in quantum dot qubits (*11*) down to the picosecond range, which still has to be reached by STM.

The advantage of the STM is that it not only probes spatial features of the decay of an excitation within a nanostructure, but it also can be used to fabricate thermodynamically metastable atomic configurations. For example, the atom-manipulation capability of the STM could be used to build a spin chain, i.e., a linear arrangement of antiferromagnetically coupled spins, as a circle of atoms on a surface. The cycling of an excited spin around this structure could then be probed by the pump-probe method measuring the spin relaxation. This experiment could be used to test the theoretically controversial prediction that spin transport in quantum-integrable spin chains—ones that can be described by exact mathematical solutions—occurs without scattering, i.e., it is ballistic (*12*). More importantly, the method developed by Loth *et al*. is not restricted to spin relaxations. It might be equally well used to follow the decay of vibrational excitations in molecules, the decay of electrically induced phase transitions, or even the decoherence dynamics of charges, spins, or vibrations.

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DEVELOPMENTAL BIOLOGY

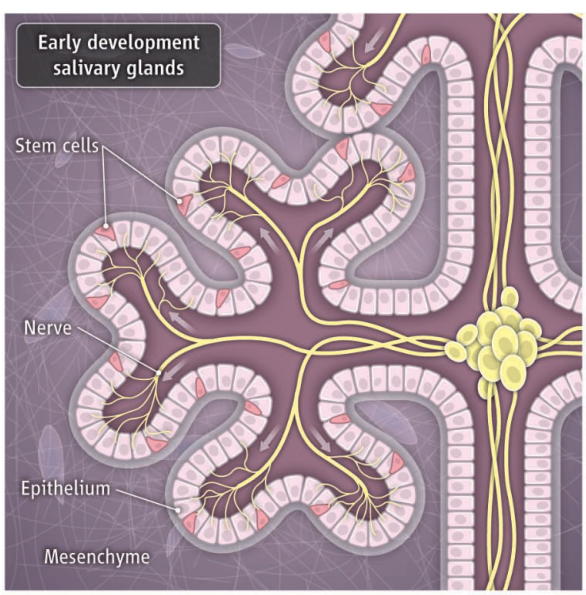
Branching Takes Nerve

Innervation is required to maintain epithelial progenitor cells that support salivary gland growth and development.

Jason R. Rock and Brigid L. M. Hogan

Nerves control salivary gland function, a connection that traces back over 100 years to the classic experiments of Ivan Pavlov, who conditioned dogs to salivate at the sound of a bell. On page 1645 in this issue, Knox *et al*. report that the effect of nerves on these glands occurs much earlier than expected (*1*). Local nerves make intimate contact with the submandibular glands—the major source of saliva in mammals—as soon they begin to develop in the embryo, and influence how the organ grows and branches. Their finding may suggest new ways to regenerate salivary glands in cancer patients exposed to head and neck irradiation, and may also be relevant to the growth and regeneration of other organ systems.

In the mouse embryo, the submandibular glands appear at mid-gestation as small epithelial buds on the floor of the oral cavity. The buds develop into the mature organ through branching morphogenesis, a process that also occurs in the lung, kidney, prostate, mammary, and lacrimal (tear) glands. This program requires reciprocal signaling between the epithelium and surrounding cells to regulate the emergence, outgrowth, and branching of new buds. Knox *et al*. show that the buds and stalks of the developing submandibular gland contain multipotent progenitor cells [marked by the expression of cytokeratin 5 ($Krt5^+$)] that fuel organogenesis. To produce an organ of the correct size and dimensions, the proliferation and differentiation of these progenitors must be tightly balanced. Rather than focus on protein factors and signaling pathways expressed in the epithelium and mesenchyme, Knox *et al*. examined the nerves that arise locally from parasympathetic ganglia and condense around the epithelial buds within a day after they first appear (see the figure). Explanted submandibular glands maintained in culture without ganglia were underdeveloped, with fewer buds compared to intact explanted glands. Similar results were observed when explanted intact glands were treated with drugs that inhibit signaling by the neurotransmitter acetylcholine, as well as when expression of an acetylcholine receptor (Chrm1) in the gland was reduced by RNA interference.



Gland progenitor cells. About 10% of the epithelial cells in the embryonic mouse submandibular salivary gland are progenitor cells ($Krt5^+$). They are located in end buds and stalks (future ducts). The parasympathetic ganglion contains nerve cells that extend along the epithelium as it undergoes branching morphogenesis.

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Knox *et al*. further determined that neuronal activity preferentially affects the epithelial progenitor cells. Explanted glands that were denervated or treated with the cholinergic inhibitor had decreased expression of genes (*Krt5*, *Krt15*, and

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Aqp3) associated with progenitor cells, and fewer and less proliferative Krt5$^+$ progenitor cells. These defects, as well as impaired branching morphogenesis, could be rescued by addition of the cholinergic agonist carbachol. Other studies in glandular epithelial cell lines showed that cholinergic stimulation triggers the release of heparin-binding epidermal growth factor (HBEGF), which binds to the EGF receptor (*2*). Knox *et al*. also found that addition of HBEGF rescued branching morphogenesis in denervated gland explants. The data support a model in which cholinergic stimulation maintains a population of proliferative Krt5$^+$ progenitors in the embryonic submandibular gland in an EGF-dependent manner.

Coordinated development of the peripheral nervous system and branched epithelial organs containing progenitor cells is also implicated in other systems, including the lung, mammary gland, and prostate. For example, stimulation of mouse embryonic lung explants with nicotine increases branching (*3*). Knox *et al*. show that inhibiting acetylcholine stimulation of explanted developing mouse ventral prostate gland leads to decreased expression of markers associated with Krt5$^+$ epithelial progenitor cells. Krt5$^+$ basal stem cells of adult mouse and human airways express the nerve growth factor receptor NGFR (p75/Tnfrsf16) (*4*), but its function is not yet clear. As well, this receptor is expressed on Krt5$^+$ basal epithelial cells of human epidermis (*5*), but to date no function has been attributed to the NGFR in epithelial development. How both nerves and growth factors coordinately interact with the salivary gland epithelium during development can now be investigated with a combination of pharmacological and genetic tools and different injury-repair models in vivo and in organ culture.

The concept of nerve-dependent organ regeneration has long been studied in the salamander, an organism with a remarkable capacity for regeneration (amputated limbs fully regenerate within several weeks). Interestingly, this process does not occur if the nerve supplying the stump is severed (*6*). But unlike the mammalian salivary gland, salamander limb regeneration is not mediated by cholinergic stimulation (*7*), but by a growth factor produced locally by the nerve (*8*).

An important question is whether the nerve–progenitor cell interaction observed by Knox *et al*. has therapeutic potential. One side effect of radiation therapy for head and neck tumors is irreparable damage to the salivary glands (*9*). This can cause xerostomia (dry mouth), a condition that severely limits postradiation quality of life and is presently incurable. However, not all damage to salivary glands is irreversible. For example, ligation of the salivary duct reverses gland atrophy in human patients as long as the autonomic innervation is intact (*10*). Thus, it might be possible to stimulate gland regeneration after irradiation by local pharmacological cholinergic stimulation of the epithelium (which harbors progenitor cells) or by promoting neurogenesis. Toward testing this hypothesis, Knox *et al*. cultured denervated lobules of adult mouse submandibular glands in the presence of the cholinergic agonist carbachol and found increased expression of Krt5 and Krt14, markers of progenitor cells. Determining whether this approach promotes organ growth and salivation in vivo will be an important next step.

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NEUROSCIENCE

Dendrites Do It in Sequences

Brain cells are exquisitely sensitive to the pattern of inputs on their dendritic branches.

Alain Destexhe

Nearly 50 years ago, Wilfrid Rall, one of the founders of computational neuroscience, proposed a mechanism to help answer one of the most basic questions in neuroscience: How do neurons detect, organize, and process the numerous chemical and electrical signals that flow from the thousands of synapses arrayed along their branching fibers (dendrites)? He proposed that the sequence in which synapses activate—specifically, whether the activation moves progressively in, along the branch toward the central cell body (soma); or out, away from the soma—could determine how the soma responds (*1*). A pattern that moved in one direction, for instance, could cause a neuron to "fire," or produce an electrical spike, whereas the soma would remain quiet if the pattern of activation moved in the opposite direction.

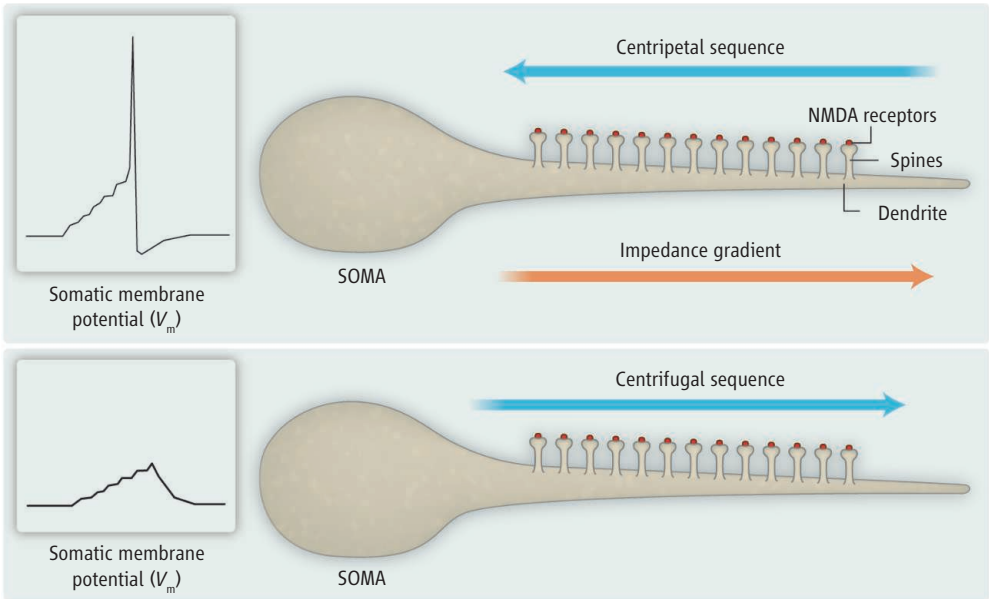
Over the past few decades, researchers have shown that individual neurons and populations process precisely timed sequences of inputs (*2*, *3*), and that "directional selectivity" appears to play a role in the sensory cortex, the part of the brain that processes information from the eyes, ears, and other sense organs (*4*). Investigators, however, had not been able to show experimentally that activating synapses in a centrifugal sequence (outward from the soma) caused a different neuronal response than activating the synapses in a centripetal (inward) sequence. Branco *et al*. (*5*) do just that in a technically impressive set of experiments described on page 1671 of this issue. Using slices of brain tissue from rats and a laser-based technique that enables stimulation of selected parts of a dendrite, they confirm Rall's idea but also reveal some surprising aspects of how directional selectivity works.

Branco *et al*. studied cortical pyramidal neurons, which are among the largest brain cells and have several kinds of dendrites, each of which carries thousands of spines, or protrusions that receive input from a synapse. Using a technique called two-photon guided laser photostimulation, they were able to release glutamate, an abundant neurotransmitter, directly onto certain spines. This enabled them to precisely control stimulation

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In and out. Neurons are sensitive to the sequence of activation of their synaptic inputs. Activating excitatory inputs on dendritic spines in a centripetal (**top**) or centrifugal (**bottom**) direction leads to different responses by the soma. One reason for this difference is that the thickness of dendrites tapers away from the soma, producing a gradient in electrical impedance. Activation sequences move with or against this gradient, and some patterns activate NMDA receptors (red), which in turn greatly amplify the excitatory signal.

in both space and time, thereby creating complex patterns of synaptic activation.

Their main finding is that the dendrites of cortical pyramidal neurons are exquisitely sensitive to the sequence of excitatory inputs, and that—as Rall predicted—centripetal activation leads to the strongest somatic response (see the figure). Rall's mechanism was not entirely correct, however, although he was remarkably forward-looking. He had postulated that directional sensitivity was entirely due to a "passive" mechanism related to the impedance, or resistance, that electrical signals encounter as they travel along a dendrite. Dendrites taper and become thinner farther from the soma. This tapering results in an "impedance gradient," with signals originating in distal dendrites (those farthest from the soma) experiencing greater impedance than those from proximal (closer) dendrites. As a result, Rall suggested that a dendrite can be seen as a "delay line" that is naturally sensitive to the location of the sequence of inputs. The main drawback of this passive mechanism, however, is that it requires very long dendrites to work efficiently. This is a problem in cortical pyramidal cells, in which up to 90% of inputs are on one type of dendrite: basal dendrites (*6*), which are too short for Rall's mechanism to work in a convincing way.

Branco *et al*. discovered an elegant alternative: Directional selectivity strongly depends on electrical voltage changes induced by synaptic activation. The authors demonstrated that a key player in sequence selectivity is the *N*-methyl-D-aspartate (NMDA) receptor, which is activated by glutamate. The receptor is voltage-dependent—it opens and closes an ion channel depending on the voltage difference between the interior and exterior of the cell (membrane potential)—and activates at depolarized, or more positive, membrane potentials (*7*, *8*). Under the right circumstances, NMDA receptors can amplify the sensitivity of a dendrite to certain sequences of synaptic activation. A sequence "favored" by the impedance gradient, for instance, also activates NMDA receptors; in contrast, inputs acting against the gradient do not achieve sufficient depolarization to activate the receptor. Indeed, blocking NMDA receptors eliminates a large part of the difference between centripetal and centrifugal activations.

The most surprising result of Branco *et al*., however, is that dendrites are not only sensitive to ordered sequences of activation on a single dendrite segment, but are also sensitive to input sequences arranged randomly, or even distributed on different dendritic branches. The authors tested many spatiotemporal patterns of spine activation and found that sequences evoking the strongest responses always corresponded to waves of centripetal activation, even when the activity was occurring on different branches. They used computational models to explore this mechanism, and found that the two basic ingredients—the impedance gradient and amplification by NMDA receptors—are sufficient to reproduce the experimental findings. This shows that the neuron can be highly selective, responding only to very specific sequences of activation from a given dendritic morphology and distribution of synaptic contacts. This mechanism may be valid for many regions of the brain, because an impedance gradient and NMDA receptors are present in most neurons.

Still, many questions remain. One is whether these results can be validated in vivo. In particular, researchers need to test whether directional sensitivity occurs with high levels of background synaptic activity, as in animals that are awake (*9*). Whether directional selectivity observed in the visual cortex has anything to do with the selectivity found by Branco *et al*. also needs to be addressed. Do dendrites process sequences involving both excitatory and inhibitory inputs? Investigators have shown that, in awake animals, some synapses inhibit a neuron's activity by influencing its action potential—the momentary change in the electrical potential on the cell's surface that occurs when it is stimulated; these inhibitory inputs may perhaps play a bigger role than excitatory inputs (*9*). Finally, it is not clear how dendritic selectivity operates under the highly nonlinear nature of voltage-dependent conductances (*10*). Could neurons regulate their intrinsic properties, position of inputs, and morphology to optimize selectivity to specific and preestablished sequences of inputs? How? Answering such questions will require new theoretical paradigms and experimental techniques.

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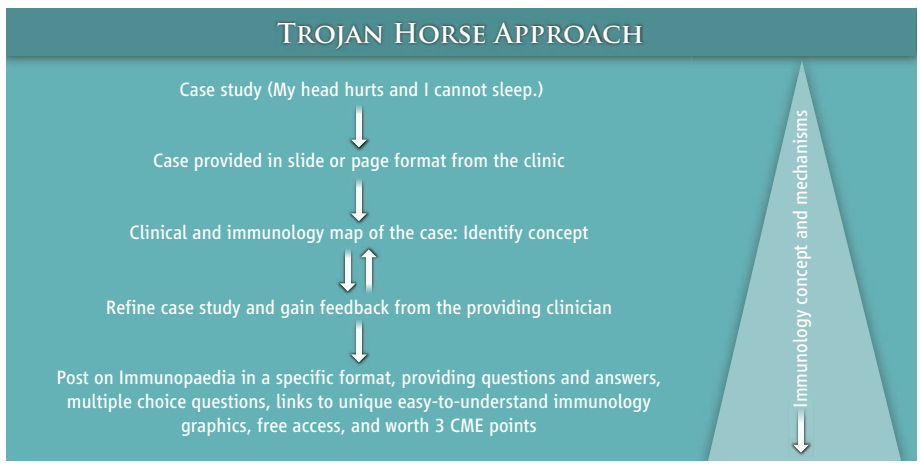
Immunology for Clinicians: A "Trojan Horse" Approach

A South African Web site imparts basic immunology information to clinicians and other health-care workers.

Clive M. Gray,[1]† Shayne Loubser,[1] Carina Kriel,[1] Monica Mercer,[1] Heather Brookes[2]

To determine how a program for learning immunology could be most useful for clinicians in South Africa, we consulted with the country's 20 leading HIV-specialist pediatricians. They told us that immunology and its underlying concepts were perceived to be complex and arcane and that there was a need for immunology to be better integrated into "real-life" clinical practice. Most saw immunology as predominantly a laboratory science with little application to clinical practice; this highlighted a gap between clinical management of patients and theoretical understanding of the etiology and immunopathogenesis of disease. We speculated that one of the possible reasons for this perception and knowledge gap is that immunology is not an independent discipline within the South African medical curriculum. Instead, it is diffusely integrated into disciplines such as pathology or internal medicine and is not specifically provided for during post-degree training.

We initially developed the project Immunopaedia (www.immunopaedia.org) to help bridge the knowledge gap between laboratory research and pediatric HIV clinical science. The rationale for Immunopaedia was based on the paradox between the void in knowledge of immunology among HIV infectious disease clinicians and the need to understand and treat the HIV epidemic in South Africa. South Africa has one of the fastest-growing HIV epidemics with over 5.5 million people living with HIV, many of whom are young children and women (*1*). The 2008 national population-based surveys estimated HIV prevalence at 16.9% (*2*). Provision of treatment and care for such a large population makes it hard for HIV clinicians to stay informed about the latest developments in infectious disease. Immunopaedia was thus created to provide an easy-to-understand summary of the effects of HIV infection on the immune system, which could facilitate improvement in clinical practice. Immunopaedia has since progressed to include more general immunology knowledge that is relevant to other disease conditions. We use a clinical case–based perspective to target interns in multiple clinical specialities. Immunopaedia is an alternative to more advanced online immunology studies that target the basic-science student (*3*). Information and educational materials are available through open access, and the site can be used as an adjunct to more formal courses or as a stand-alone learning tool.



Trojan Horse approach. Immunopaedia uses a case study to lure clinicians into learning immunology. Shown are the steps followed for each case before posting on the Web site. The case is used to underpin an immunology concept.

Our main method is what we have termed the "Trojan Horse" approach. Derived from the Greek *Iliad*, "Trojan Horse" has come to mean any ploy or scheme used to insinuate a rival into a securely protected stronghold. We use clinical case studies as our Trojan Horse to tempt clinicians, most of whom consider immunology to have little application to clinical needs, to engage with immunological concepts relevant to diagnosis and treatment (see the figure, above). We explain the key immunological points related to the cases by means of a series of graphics.

The Web site consists of three key components: Clinical Cases, Immunology Learning, and Treatment and Diagnostics. At present, 34 clinical cases are available on the site. In addition to HIV, clinical cases span topics such as hypersensitivity, tuberculosis (TB) immunopathology, primary and secondary immunodeficiencies, drug responses, gastrointestinal disorders, autoimmunity, and malignancies. Each of our cases is used to examine an immunological concept that leads to greater understanding of the human immune system. For example, a case of a 14-year-old boy presenting with severe hip pain allowed us to discuss ankylosing spondylitis and to explore the concept of mimicry and the "arthritogenic peptide" hypothesis. The case of repeated apnea and infections in a premature infant allowed us to highlight "physiological immunodeficiency" caused by impaired humoral and cellular responses in premature infants, a situation that leaves such infants vulnerable to both viral and bacterial pathogens. Another presenting case was an 8-month-old boy with recurrent infections. We explored the most likely hypothesis, that the mother had a primary HIV-1 infection during pregnancy in the third trimester and that the child was infected perinatally before maternal seroconversion. These are real-life cases, and in the last-mentioned case, as often in real life, the underlying immunological problem may not be clear.

Each case discussion provides, in consecutive windows, patient presentation, history, differential diagnosis, examination, investigations, discussion, treatment, and a final outcome. Related case studies are cross-referenced. Users then evaluate their

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knowledge and understanding of the concepts in the case study by means of questions accompanied by explanations. To earn the full quota of continuing medical education (CME) points, the user can complete five multiple choice questions for each case study that are freely available but require log-in information. Integral to our approach with each case study is the use of clear graphics to explain the immunological basis and mechanisms underlying the condition. The graphics can be downloaded freely as a PDF and used for offline reference or for further teaching tools.

The second key component, Immunology Learning, is divided into three main areas: "Immunology" (dealing with the basics), "Childhood Diseases and Vaccinations" (dealing with hypersensitivity reactions and immune reconstitution, for example), and "Infections" (dealing with a range of infections from rickettsia to Guillain-Barré syndrome).

The third key component, Treatment and Diagnostics, covers information related to TB and antiretroviral (ARV) drugs, modes of drug action, the HIV life cycle, and specific guidelines for treatment. The "Diagnostic Tools" section provides laboratory assay information, such as enzyme-linked immunosorbent assays (ELISAs), polymerase chain reactions (PCRs), and flow cytometry.



Workshop participants evaluate case study material. Participants of workshops come from tertiary teaching hospitals within South Africa and include interns specializing in pediatrics, pathology, and internal medicine.

Immunopaedia provides links to other Web sites that focus on medical and general aspects of HIV, pediatrics, and treatment guidelines. The design of the Web site is such that the user can easily find a source of information in the form of a case study, a graphic, or a scientific article on specific immunological conditions. In addition, breaking scientific news on clinical immunology is posted on the home page and is updated daily. Registered users are sent a monthly newsletter that coincides with a monthly clinical case study posting.

For the clinical case component, medical practitioners submit case studies from their clinics. Our team of clinical and immunology experts reviews and modifies these case studies for presentation on the Web site (see the first figure). The rationale behind this approach is that case studies from, or closely related to, real situations are effective learning tools (*4*). The case-study approach is a form of experiential learning; it integrates practice, knowledge, and skills that further equip clinicians in their professional work (*5*). As the user reads through the case study on the site, dividing the case study into stages in separate windows, the user is challenged to think about and predict a possible diagnosis based on the clinical evidence he or she has just read. This process is similar to the way a clinician might operate on the job, and the approach fosters lateral and critical thinking, as well as self-guided learning (*6*).

Immunopaedia also includes a workshop component as a follow-up to the online material (see the second figure). We use the workshops to evaluate our new case-study material before posting it on the Web site, and our teaching team travels to medical schools within South Africa to hold 3-hour sessions for medical interns who are specializing in pediatrics, pathology, or internal medicine. The aim of the workshops is to create greater awareness and increased use of Immunopaedia as a learning site for clinical immunology.

The monthly average number of unique users was 320 in 2007; 1518 in 2008; 2326 in 2009; and 1723 in 2010 (through June). Since the site first went live, we have received a total of 87,550 visitors, of which 11,520 have spent more than 5 minutes on the site. Each user who completes a case study earns three CME points, and in total, we have awarded 699 CME points over 3 years. We currently have 892 registered users.

Future directions for Immunopaedia involve the expansion of our user base to include point-of-care clinicians for the ARV drug roll-out in South Africa. We will use clinical case studies to highlight common diagnostic decision points as a mechanism to educate clinicians on laboratory tools and interpretation of results.

Immunopaedia is an immediate source of information for professionals and represents an effective means for learning and dissemination of immunology information. Our aim is to integrate immunology into clinical options for patient management.

About the authors



Clive Gray, founder of Immunopaedia, has a Ph.D. in immunology and conducts research on HIV/AIDS in South Africa. He received the Elizabeth Glaser Pediatric AIDS Foundation International Leadership Award, which has allowed the development of Immunopaedia. **Shayne Loubser** writes the immunology materials and produces the computer-generated graphics for each case study. **Carina Kriel** is the curator and coordinator of the Web site and oversees all administrative aspects. **Monica Mercer** (not pictured) holds an M.D. and is a clinical materials writer and leads the development of educational materials. **Heather Brookes** (not pictured) has a Ph.D. in language, literacy, and culture and is a consultant for Internet learning. From left to right: Shayne Loubser, Clive Gray, and Carina Kriel.

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INTERNATIONAL

Singapore Statement Urges Global Consensus on Research Integrity

Though they come from different countries and cultures, researchers worldwide should take responsibility for the trustworthiness of their work and recognize ethical obligations to society, says a new statement from the 2nd World Conference on Research Integrity, co-sponsored by AAAS.

The Singapore Statement on Research Integrity, released 22 September, lists 16 responsibilities for researchers. Scientists should disclose any conflicts of interest, give proper credit to their collaborators, and report irresponsible research practices, it says. The statement also urges research institutions and journals to develop procedures for responding to allegations of misconduct.

More than 300 delegates from 51 countries contributed to the succinct one-page statement (see www.singaporestatement.org). Although research regulations vary internationally, the delegates concluded that "there are also principles and professional responsibilities that are fundamental to the integrity of research wherever it is undertaken."

Policy-makers, university leaders, publishers, and government ministers first drafted the statement at the conference, held 21 to 24 July in Singapore. The conference was supported by science associations from China, Japan, South Africa, Saudi Arabia, Australia, South Korea, the United Kingdom, and the United States. Mark S. Frankel, director of the AAAS Scientific Freedom, Responsibility and Law Program, and Gerald Epstein, director of the AAAS Center for Science, Technology and Security Policy, gave presentations at the meeting. Senior Program Associate Deborah Runkle was a cochair of the conference's session on digital plagiarism.



Mark S. Frankel



Gerald Epstein

After the First World Conference on Research Integrity, held in 2007 in Lisbon, Portugal, the Singapore attendees sought a set of "international norms and standards related to research integrity that would accommodate national differences," said Frankel.

The harmonization of these standards is part of a larger effort by AAAS to support the international integration of scientific values. Another example: In June, after 3 years of top-level discussion, the China Association for Science and Technology and AAAS established a joint steering committee to coordinate work on scientific ethics.

Seeking an international integrity agreement is one way to pursue harmonization, said Epstein. "Although many different groups have different conceptions of what a code of conduct should focus on," he explained, "there isn't any culture in which making up data is good."

Epstein was pleased that the statement included an obligation to consider the societal benefits and risks of research. "There are those who think scientists have no business extrapolating the social context of their work," he said, "but that's not a view that I think the rest of society would be very comfortable with."

Frankel said the recent "Climategate" controversy, fueled by e-mails hacked from United Kingdom researchers and a handful of errors discovered in the massive 4th Assessment Report of the Intergovernmental Panel on Climate Change, demonstrated how public trust in science can be weakened by suspicions of irresponsible advocacy.

"It's a problem for all scientists," said Frankel. "When do scientists cross the line from being an independent source of valued information to designing or using their research to support some preconceived policy preference?"

The Singapore Statement can guide governments, research institutions, and professional societies as they develop their own integrity standards, he noted.

"At AAAS, we believe that high ethical standards go hand-in-hand with quality research," Frankel said. "The freedom to do research cannot be separated from the ethical responsibilities that researchers have to the integrity of their research and the larger society that supports them." —*Becky Ham*

EDUCATION

Undergrad S&T Classes: Innovation and Excellence

At Syracuse University, students learn computer security by studying hackers' tricks. At Western Washington University, faculty and staff have developed a virtual laboratory that allows students thousands of miles away to use the university's advanced scientific equipment. At the University of Mississippi, research has shown that case studies are a powerful learning tool in biology classes.

Those innovations in undergraduate education are among 17 highlighted in a recent report on the U.S. National Science Foundation program Transforming Undergraduate Education in Science, Technology, Engineering, and Mathematics (TUES). AAAS administered the program's 2008 meeting and developed the ensuing report, "New Challenges, New Strategies: Building Excellence in Undergraduate STEM Education," which was delivered this month to members of Congress and Capitol Hill staff.

"We're looking for things that you can hold up and say, 'Yes, there are things that we can do to provide a better science experience for people,'" said Shirley Malcom, head of Education and Human Resources at AAAS. "We have to think: What are they going to walk away with as the headline of science in their lives as they go on to be journalists or judges or whatever they want to be?"

To see the TUES report, visit www.aaas.org/go/ccli09/. —*Meagen Voss contributed to this story.*

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Reaction-Diffusion Model as a Framework for Understanding Biological Pattern Formation

Shigeru Kondo[1]* and Takashi Miura[2]

The Turing, or reaction-diffusion (RD), model is one of the best-known theoretical models used to explain self-regulated pattern formation in the developing animal embryo. Although its real-world relevance was long debated, a number of compelling examples have gradually alleviated much of the skepticism surrounding the model. The RD model can generate a wide variety of spatial patterns, and mathematical studies have revealed the kinds of interactions required for each, giving this model the potential for application as an experimental working hypothesis in a wide variety of morphological phenomena. In this review, we describe the essence of this theory for experimental biologists unfamiliar with the model, using examples from experimental studies in which the RD model is effectively incorporated.

Over the past three decades, studies at the molecular level have revealed that a wide range of physiological phenomena are regulated by complex networks of cellular or molecular interactions (*1*). The complexity of such networks gives rise to new problems, however, as the behavior of such systems often defies immediate or intuitive understanding. Mathematical approaches can help facilitate the understanding of complex systems, and to date, these approaches have taken two primary forms. The first involves analyzing every element of a network quantitatively and simulating all interactions by computation (*1*). This strategy is effective in relatively simple systems, such as the metabolic pathway in a single cell, and is extensively explored in the field of systems biology. However, for more complex systems in which spatiotemporal parameters take on importance, it becomes almost impossible to make a meaningful prediction. A second strategy, one that includes simple mathematical modeling in which the details of the system are omitted, can be more effective in extracting the nature of the complex system (*2*). The reaction-diffusion (RD) model (*3*) proposed by Alan Turing is a masterpiece of this sort of mathematical modeling, one that can explain how spatial patterns develop autonomously.

In the RD model, Turing used a simple system of "two diffusible substances interacting with each other" to represent patterning mechanisms in the embryo and found that such systems can generate spatial patterns autonomously. The most revolutionary feature of the RD model is its introduction of a "reaction" that produces the ligands (morphogens). If "diffusion" alone is at work, local sources of morphogens are needed to form the gradient. In such cases, the positional information made by the system is dependent on the prepattern (Fig. 1, A and B). By introducing the reaction, the system gains the ability to generate various patterns independent of the prepattern (Fig. 1C). Unfortunately, Turing died soon after publishing this seminal paper, but simulation studies of the model have shown that this system can replicate most biological spatial patterns (*4*–*6*). Later, a number of mathematical models (*4*) were proposed, but most followed Turing's basic idea that "the mutual interaction of elements results in spontaneous pattern formation." The RD model is now recognized as a standard among mathematical theories that deal with biological pattern formation.

However, this model has yet to gain wide acceptance among experimental biologists. One reason is the gap between the mathematical simplicity of the model and the complexity of the real world. The hypothetical molecules in the original RD model have been so idealized for the purposes of mathematical analysis that it seems nearly impossible to adapt the model directly to the complexity of real biological systems. However, this is a misunderstanding to which experimental researchers tend to succumb. The logic of pattern formation can be understood with simple models, and by adapting this logic to complex biological phenomena, it becomes easier to extract the essence of the underlying mechanisms. Genomic data and new analytic technologies have shifted the target of developmental research from the identification of molecules to understanding the behavior of complex networks, making the RD model even more important as a tool for theoretical analysis.

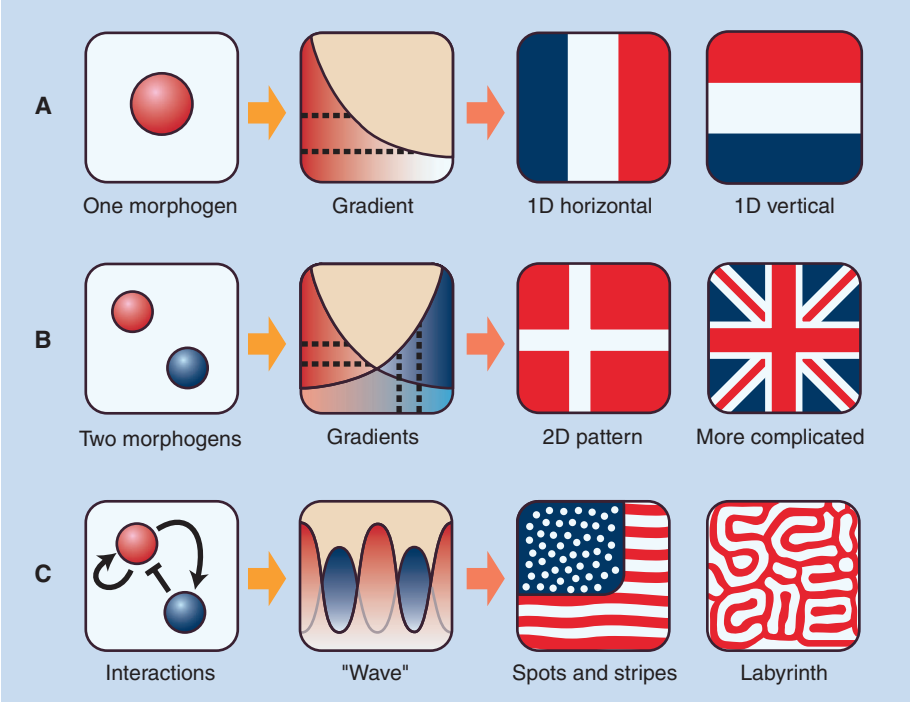


Fig. 1. Schematic drawing showing the difference between the morphogen gradient model and Turing model. (**A**) A morphogen molecule produced at one end of an embryo forms a gradient by diffusion. Cells "know" their position from the concentration of the molecule. The gradient is totally dependent on the prepattern of the morphogen source (boundary condition). (**B**) Adding a second morphogen produces a relatively complex pattern; but with no interactions between the morphogens, the system is not self-regulating. (**C**) With addition of the interactions between the morphogens, the system becomes self-regulating and can form a variety of patterns independent of the prepattern. [Art work by S. Miyazawa]

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In this review, we describe the RD model and its experimental applications, addressing some of the points biologists tend to question. We hope many biologists gain an appreciation for this beautiful theory and take advantage of it in their experimental work.

The Original Turing Model

At the beginning of his original paper (*3*), Turing stated that his aim was to show that the combination of known physical elements is sufficient to explain biological pattern formation. The elements selected by Turing were a theoretical pair of interacting molecules diffused in a continuous field. In his mathematical analysis, Turing revealed that such a system yields six potential steady states, depending on the dynamics of reaction term and wavelength of the pattern [Fig. 2A and Supporting Online Material (SOM) Text 1-1]. In case I, the system converges to a stable and uniform state. In case II, a uniform-phase oscillation of the morphogen concentration occurs. Such phase unification is seen in such systems as circadian rhythms (*7*) and the contraction of heart muscle cells (*8*). In case III, the system forms salt-and-pepper patterns, such as are made when differentiated cells inhibit the differentiation of neighboring cells. This is seen, for example, with differentiated neuroprogenitor cells in the epithelium of *Drosophila* embryos (*9*). Case IV represents an unusual state in which a pattern of the sort made in case III oscillates. No examples of this have been identified in a developmental system. In case V, a traveling wave is generated. Biological traveling waves caused by this mechanism include the spiral patterns formed by the social amoeba *Dictyostelium discoideum* on aggregation (*10*), and the wave of calcium ions that traverses the egg of the frog *Xenopus laevis* on sperm entry (*11*).

In case VI, stationary patterns are made. The finding of this type of wave is the major achievement of Turing's analyses, and these are usually referred to as Turing patterns. A Turing pattern is a kind of nonlinear wave that is maintained by the dynamic equilibrium of the system. Its wavelength is determined by interactions between molecules and their rates of diffusion. Such patterns arise autonomously, independent of any preexisting positional information. [The supporting materials contain an expanded explanation of the RD model for biologists who are unfamiliar with the mathematical description (SOM Text S1-1), as well as a mathematical explanation of how the periodic pattern arises in the system (SOM Text S1-2).] This property makes it possible to explain how patterns arise precisely in, for example, fertilized oocytes, which present little in the way of positional information. The ability of Turing patterns to regenerate autonomously, even after experimentally induced disturbances, is also important and of great utility in explaining the autonomy shown by pattern-forming developmental processes (*4*, *6*). In addition, through the tuning of parameters and boundary conditions, the system underlying Turing pattern formation can generate a nearly limitless variety of spatial patterns. (Figure 2B shows representative two-dimensional patterns made by simulations with the RD model.) The intricate involutions of seashells (*5*), the exquisite patterning of feathers (*12*), and the breathtakingly diverse variety of vertebrate skin patterns (*13*) have all been modeled within the framework of the Turing model (Fig. 2C). The remarkable similarity between prediction and reality in these simulations points strongly to Turing mechanisms as the underlying principle in these and other modes of biological patterning. (User-friendly simulation software is available as supporting online material; a manual of the software is in SOM Text 1-3.)

Compatibility of RD and Gradient Models

Experimental biologists may recall the "gradient model," which has been quite effective in explaining pattern-formation events (*14*). In the classic gradient model, the fixed source of the morphogen at a specific position provides positional information (*14*) (Fig. 1). Although the assumption of a morphogen source appears to be different from the assumptions of the RD model, it can be introduced into the RD model quite naturally as a "boundary condition." In other words, the classic morphogen gradient model can be thought of as the specific case of the RD model in which the reaction term is removed. In many simulation studies, such boundary conditions are used to make the pattern more realistic (*6*). Recent experimental studies of morphogen gradients have shown that the precision and the robustness of the gradient are secured by the interactions of molecular elements (*15*). To model such situations, the authors used a mathematical framework that is essentially identical to that of the RD model. Both reaction and boundary conditions are essential to understanding complex real systems, and the RD model is useful for modeling such cases.

Applying the "Simple" RD Model to a Complex Reality

The hypothetical molecules in the original RD model (*3*) are idealized for the purposes of mathematical analysis. It is assumed that they control their own synthesis and that of their counterparts, and diffuse quickly across spaces that would be divided by cellular membranes. Obviously, it is quite difficult to apply such a model directly to complex living systems.

Concerted efforts to align theoretical models to real-world systems, however, have begun to bear fruit, pointing to a much broader range of situations in which the general principles underlying the Turing model might apply. Gierer and Meinhardt (*16*, *17*) showed that a system needs only to include a network that combines "a short-range positive feedback with a long-range negative feedback" to generate a Turing pattern. This is now accepted as the basic requirement for Turing pattern formation (*14*, *16*). This refinement leaves the types and numbers of reacting factors unspecified, making it much simpler to envision systems that might fit the requirements.

The interacting elements need not be limited to molecules, or even to discrete entities; a circuit of cellular signals will do just as well (*18*). There is also no need for the stimulus to be provided via diffusion; other modes of transmission can achieve the same end result. Theoretical modeling has shown that a relayed series of direct cell-to-cell signals can form a wave having properties similar to one formed by diffusible factors (*19*). Other forms of signaling, including chemotactic cell migration (*20*), mechanochemical activity (*21*), and neuronal interactions [as in the Swindale model (*22*) of ocular dominance stripe formation], are also capable of forming Turing-like patterns. For all of these systems, a similar periodic pattern is formed when the condition of "short-range positive feedback with long-range negative feedback" is satisfied.

Why systems represented by apparently different equations behave similarly, and how much the capacity for pattern forming differs among them, are the important subjects from the mathematics perspective. But if the dynamics of the systems are nearly the same, experimental researchers can select any of the models as their working hypothesis. In the case of fish skin patterning, although experiments apparently suggest the involvement of a nondiffusing signal transduction mechanism, the simplest RD model can predict the movement of the pattern during fish growth (*23*) and the unusual patterns seen in hybrid fish (*24*).

Finding Turing Patterns in Real Systems

During embryogenesis, a great variety of periodic structures develop from various nonperiodic cell or tissue sources, suggesting that waves of the sort generated by Turing or related mechanisms may underlie a wide range of developmental processes. Using modern genetic and molecular techniques, it is possible to identify putative elements of interactive networks that fulfill the criteria of short-range positive feedback and long-range negative feedback, but finding the network alone is not enough. Skeptics rightly point out that just because there is water, it doesn't mean there are waves. No matter how vividly or faithfully a mathematical simulation might replicate an actual biological pattern, this alone does not constitute proof that the simulated state reflects the reality. This has been another major hurdle in identifying compelling examples of Turing patterns in living systems. The solution, however, is not so complicated; to show that a wave exists, we need to identify the dynamic properties of the pattern that is predicted by the computer simulation. Experimental demonstrations have focused on pattern formation in the skin, because the specific characteristics of Turing patterns are more evident in two dimensions than in one.

Turing Patterns in Vertebrate Skin

Observation of the dynamic properties of Turing patterns in nature was made by Kondo and Asai

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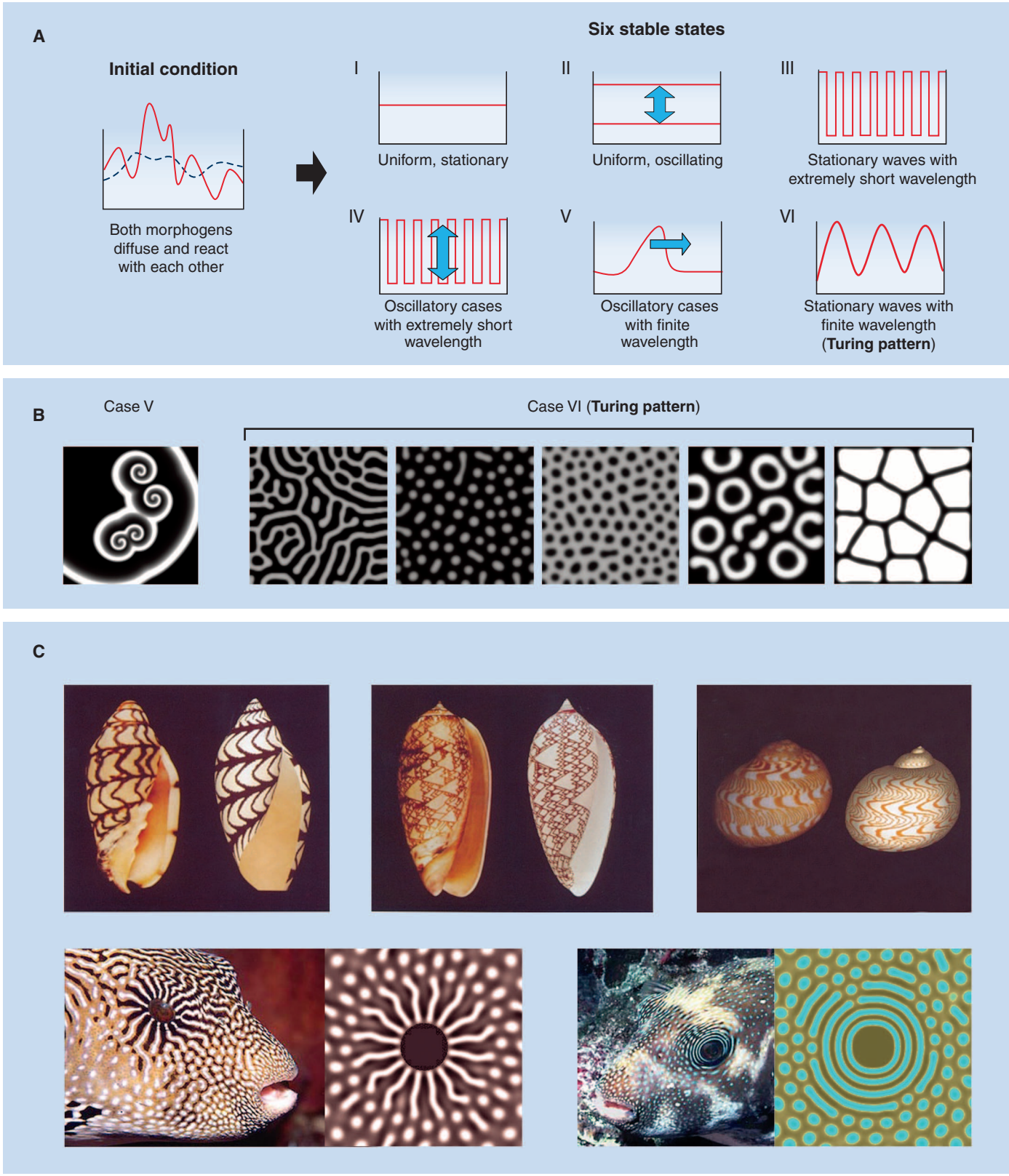


Fig. 2. Schematic drawing showing the mathematical analysis of the RD system and the patterns generated by the simulation. (**A**) Six stable states toward which the two-factor RD system can converge. (**B**) Two-dimensional patterns generated by the Turing model. These patterns were made by an identical equation with slightly different parameter values. These simulations were calculated by the software provided as supporting online material. (**C**) Reproduction of biological patterns created by modified RD mechanisms. With modification, the RD mechanism can generate more complex patterns such as those seen in the real organism. Simulation images are courtesy of H. Meinhardt [sea shell pattern (*5*)] and A. R. Sandersen [fish pattern (*13*)]. Photos of actual seashells are from Bishougai-HP (http://shell.kwansei.ac.jp/~shell/). Images of popper fish are courtesy of Massimo Boyer (www.edge-of-reef.com).

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in a study of horizontal stripes in the tropical fish, *Pomacanthus imperator* (*25*). They have recently shown that this dynamic nature is shared by many fish species, including the well-established model organism, zebrafish. Although zebrafish stripes may appear to be stationary, experimental perturbation of the pattern triggers slow changes (*23*). Following laser ablation of pigment cells in a pair of black horizontal stripes, the lower line shifts upward before stabilizing in a Bell-like curve (Fig. 3A) (*23*). As a result, the spatial interval between the lines is maintained, even when their direction changes. This striking behavior is predicted by simulation (Fig. 3B).

Fortunately, the zebrafish is amenable to a variety of experimental approaches that may lead to the identification of the circuit of interactions that generates these patterns (*26*). Work to date has shown that the skin patterns of this fish are set up and maintained by interactions between pigmented cells (*26*). Nakamasu worked out the interaction network among the pigment cells. Although the shape of the network is different from that of the original Turing model, it fits the short-range positive, long-range negative feedback description (*18*). (The mutual inhibition between black and yellow cells behaves as a positive feedback loop, as the expansion of black cells weakens their counterpart.) Identification of the genetic factors involved in the zebrafish pigmentation is under way (*26*), and it is hoped that this will clarify the details of the signaling network beneath the waves in the skin of fish, and potentially all vertebrates. Many similar surface patterns are seen in invertebrates and plants. We suppose that in these cases an essentially similar mechanism (RD mechanism) is involved, although their molecular basis may be different.

Other well-studied examples include the regular disposition of feather buds in chick (*27*), and of hair follicles in mice (*28*). Jung *et al*. showed that the spatially periodic pattern of feather buds regenerates even when the skin is recombined from dissociated cells (*27*). In the case of mouse hair follicles, alteration of the amount of putative key factors changes the pattern in a manner predicted by computer simulation. Sick *et al*. used overexpression and inhibition of Wnt and Dkk in fetal mouse to study how such perturbations might affect the patterning of follicle formation (*28*). By simulation, they first predicted that a ringed pattern of Wnt gene expression that does not occur in nature would result if ectopic production of a Wnt protein were to be controlled appropriately, and then confirmed this experimentally. They suggested that Wnt serves as a short-range activator, and Dkk as a long-range inhibitor, in this system (*28*). This pair of factors functions in various patterning processes as well, making this a critically important result for the intimations it provides of a wider role for Turing patterns in development (Fig. 4). Interestingly, the growth of new hair relying on interactions between neighboring follicles proceeds even in adult mice, and in one case a mutant was identified in which a traveling wave of hair formation gradually moved across the skin over the life of animals carrying this mutation (*29*). Plikus *et al*. (*29*) have shown how the factors FGF (fibroblast growth factor) and BMP (bone morphogenetic protein) function to generate the traveling wave (case V in the Turing model).

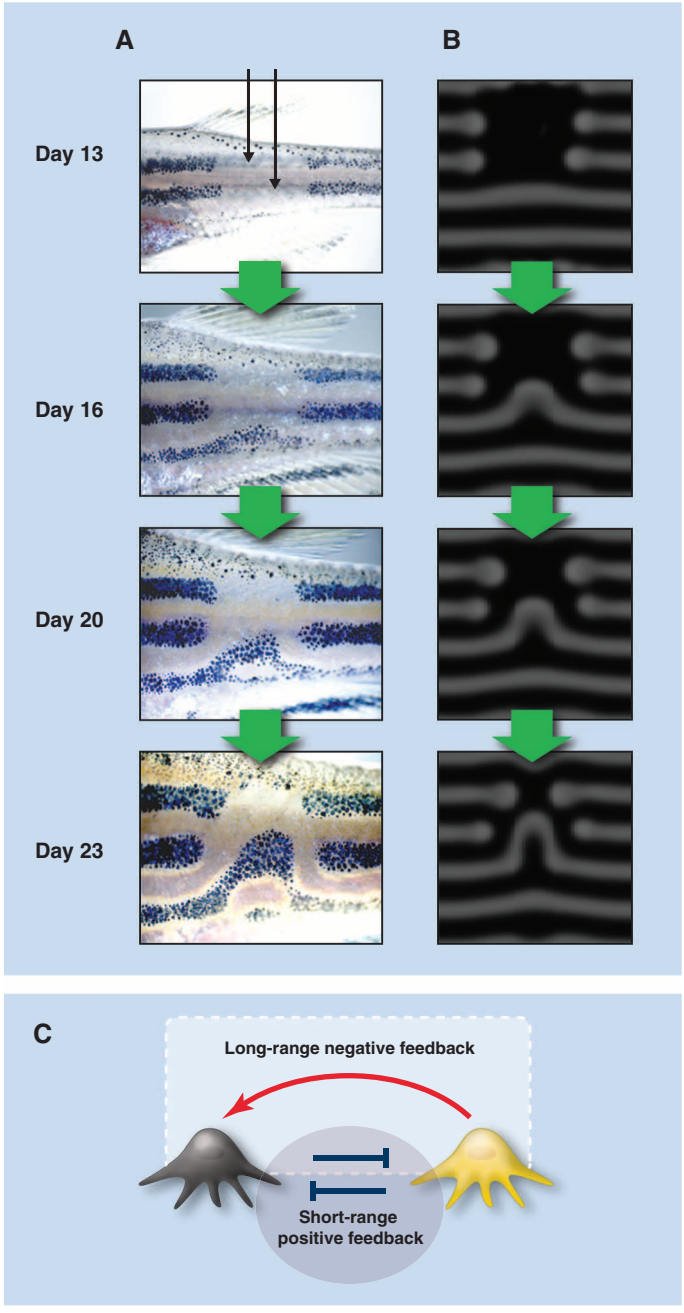


Fig. 3. Movement of zebrafish stripes and the interaction network among the pigment cells. The pigment pattern of zebrafish is composed of black pigment cells (melanophores) and yellow pigment cells (xanthophores). The pattern is made by the mutual interaction between these cells. (**A**) Melanophores in the two black stripes were ablated by laser, and the process of recovery was recorded. (**B**) Results of simulation by the Turing model. (**C**) Interaction network between the pigment cells deduced by experiments. The red arrow represents a long-range positive (enhancing) effect, whereas the blue line with the end bar represents a short-range negative (inhibitory) effect. A circuit of two negative interactions functions as a positive feedback.

Other Potential Turing-Driven Developmental Phenomena

Establishment of right-left asymmetry in vertebrates is triggered by the unidirectional rotation of cilia at the node, followed by the interaction of Nodal and Lefty that amplify and stabilize faint differences in gene expression (*30*, *31*). Nodal enhances both its own expression and that of Lefty, and Lefty inhibits the activity of Nodal. The fact that Lefty spreads further than Nodal suggests that the inhibitory interaction propagates more quickly than does its activating counterpart, which as we have seen, indicates that this system fulfills the fundamental requirements for Turing pattern formation (*30*) (Fig. 4).

In vertebrate limb development, precartilage condensation, which is later replaced by skeletal bone, occurs periodically along the anterior-posterior axis of the distal tip region. Because this patterning occurs without any periodic prepattern, the Turing model has long been suggested to describe the underlying mechanism (*32*). In this system, transforming growth factor–β (TGF-β) has been invoked as a candidate for the activator molecule (*33*). TGF-β can stimulate its own production and trigger precartilage condensation, and the sites of incipient condensation exert a laterally acting inhibitory effect on chondrogenesis. Although no candidate inhibitor has been identified, an interaction network comprising TGF-β function and precartilage condensation may satisfy the short-range activation and long-range inhibition criteria (*33*). Miura and Shiota have shown that nearly periodic spatial patterns of chondrogenesis occur in the culture of dissociated limb cells in vitro,

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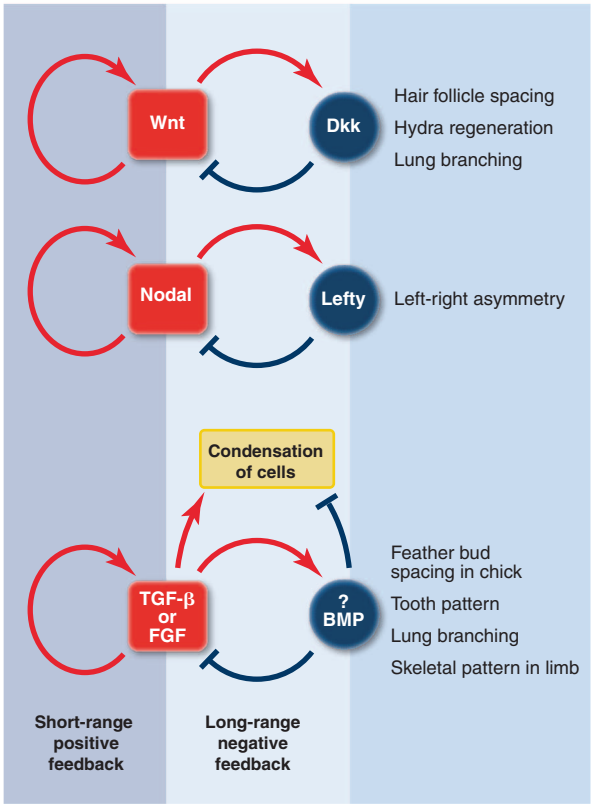


Fig. 4. Possible networks of protein ligands may give rise to Turing patterns in the embryo. Shown above are candidates for the RD mechanism proposed by molecular experiments. For a detailed explanation of each network, refer to the text and the articles listed in the references. In all these cases, the network is identical to that of the activator-inhibitor model proposed by Gierer and Meinhardt (*17*). Condensation of cells by migration into a local region causes sparse distribution of cells in a neighboring region. This can also function as long-range inhibition. (Note that the involvement of the RD mechanism in some of the phenomena above has not been fully accepted by experimental researchers.)

and that the addition of TGF-β changes the pattern in a manner consistent with the predictions of Turing's model (*34*).

Wnt and Dkk are also essential for lung branching in vertebrates (*35*) and play a key role in head regeneration in *Hydra* (*36*), in which involvement of the Turing mechanism has long been suggested by theoretical and experimental studies. Involvement of the same molecules does not directly prove that the same dynamic mechanism is at work. However, because they constitute the self-regulated system that is robust to artificial perturbations, it is reasonable to expect that the Turing mechanism may underlie these phenomena as it does those in the skin. Watanabe *et al*. have shown that signal transduction via the gap junction (connexin41.8) plays a key role in pigment pattern formation in zebrafish (*37*). Loss of function of the connexin gene (*leopard*) reduces the spatial periodicity and changes the pattern from stripes to spots (*37*). A mutation in another gap junction gene, *connexin43*, shortens each fin ray, resulting in shortened fins (*38*). Although the detailed molecular mechanism underlying this phenomenon has yet to be elucidated, it is possible that the same mechanism functions in other processes of animal development. The above examples are by no means an exhaustive list of the candidates currently being examined as potential biological Turing patterns. More detailed discussions are provided in (*4*) and (*6*).

Identification of the specific dynamics of the RD system is critical to showing the applicability of the Turing mechanism to the formation of a given pattern. In the case of pigment pattern formation, it was possible to disturb the pattern and observe the process of regeneration. In most other systems, such observation is complicated because experimental perturbations may be lethal. This is one reason why it is difficult to demonstrate RD activity in some biological systems. (In SOM Text 1-4, we have summarized the important points for researchers to keep in mind when they use the RD model as the working hypothesis.) However, recent technological advances in imaging technologies are assisting such studies. Moreover, the artificial generation of Turing patterns in cell culture should be possible in the near future as the result of synthetic biology (*39*). Turing was born in 1912 and published his RD model in 1952. Although he was unable to witness the impact of his hypothesis on the work of contemporary biologists, we are hopeful that with an increased acceptance among experimental biologists of the principles he first elucidated, we will see Turing's mechanism take its place as a model for the understanding of spatial pattern formation in living systems.

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Supporting Online Material

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Eocene Patagonia Fossils of the Daisy Family

V. D. Barreda,[1,2]* L. Palazzesi,[1,2] M. C. Tellería,[2,3,4] L. Katinas,[2,5] J. V. Crisci,[2,3,5] K. Bremer,[6] M. G. Passalia,[2,7] R. Corsolini,[8] R. Rodríguez Brizuela,[1] F. Bechis[2,9]

Asteraceae, the family of daisies, sunflowers, and dandelions, is the most diverse family of flowering plants on Earth in terms of numbers of genera and species and widespread distribution. Its most prominent feature is the capitulum, a cluster of sessile flowers crowded onto a receptacle and an efficient reproductive unit that might have provided a larger pollinator target.

Asteraceae and their most closely related families (Goodeniaceae and Calyceraceae in Asterales) have a meager fossil record (*1*). Although plant megascopic preservation is uncommon (*2*), fossil pollen grains are widely recorded, particularly during the Neogene (*1*, *3*). The oldest pollen records are assigned to subfamilies Carduoideae and Mutisioideae from the Paleogene of Africa, Australia, and southern South America (*4*–*7*).

We describe here a fossil capitulum unequivocally assignable to Asteraceae associated with dispersed pollen grains in Middle Eocene [47.5 × 10^6 years ago (Ma)] rocks from northwestern Patagonia, southern South America.

The fossil (Fig. 1) consists of a loosely cymose capitulescence with long pedunculate capitula. At least the central capitulum appears to be fully mature. It possesses several series of protective bracts (phyllaries), the basalmost decurrent onto the long peduncle. Intermediate bracts are widely oblong, with an apparent rounded apex. Flowers (~80) are relatively large, with a developed ligule or lip. Slender projections like hairs (pappus) are present among flowers. Pollen grains are tricolporate, echinate, with well-developed spines and a bilayered acaveate exine. These morphological features fit well with fossil *Mutisiapollis telleriae* from the Oligocene of Patagonia and in part with *Mutisiapollis viteauensis* from Africa (*4*, *5*, *7*).

Several features indicate a close relationship with extant Asteraceae (table S1). The particular arrangement of the capitula in a cymose capitulescence, the many-flowered capitula with multiseriate-imbricate involucral bracts, the pappus-like structures, and the pollen morphology represent a unique combination of features of Asteraceae. The acaveate exine and the conspicuous spines with uneven arrangement characterize pollen of most members of Stifftieae and Wunderlichioideae centered in the Guayana Highland, some Gochnatieae (Mutisioideae *sensu lato*), and some Dicomae and Oldenburgieae (Carduoideae) (*8*–*10*) and exclude the subfamily Barnadesioideae.

The Eocene paleoclimatic scenario inferred for the fossil-bearing locality suggests warm [mean annual temperature (MAT) estimate 19.2° ± 2.4°C] and humid [mean annual precipitation (MAP) estimate 2000 to 2500 mm] climatic conditions, supporting a diverse subtropical vegetation on the northwestern Patagonian region (*11*), perhaps with some patches of drier areas (*12*). The identification of the asteracean fossil provides an important addition to the diversity of the Eocene subtropical flora. Members of Asteraceae probably inhabited dry or transitional zones between humid and dry areas, as the majority of current species do.

The Palaeogene record of Asteraceae is so sparse that the discovery of one new fossil can have a substantial effect on known time ranges in the phylogenetic tree. Fossils from unequivocally Middle Eocene deposits of Patagonia allow us to assume that the divergence age of Mutisioideae s. l. and Carduoideae from Barnadesioideae is at least 47.5 Ma. This age is older than that estimated by the last molecular clock for the Mutisioideae s. l.–Carduoideae origin (Late Eocene, 38 to 42 Ma) (*13*) but may be consistent with the age of origin estimated for the whole family (Early Eocene, 50 Ma) (*14*).

This finding of Eocene fossils with Mutisioideae-Carduoideae affinities in southern South America, together with those reported from Africa (*4*, *5*) and Australia (*6*), suggests that an ancestral stock of Asteraceae may have formed part of a geoflora widespread across southern Gondwana before the establishment of effective dispersal barriers within this landmass.

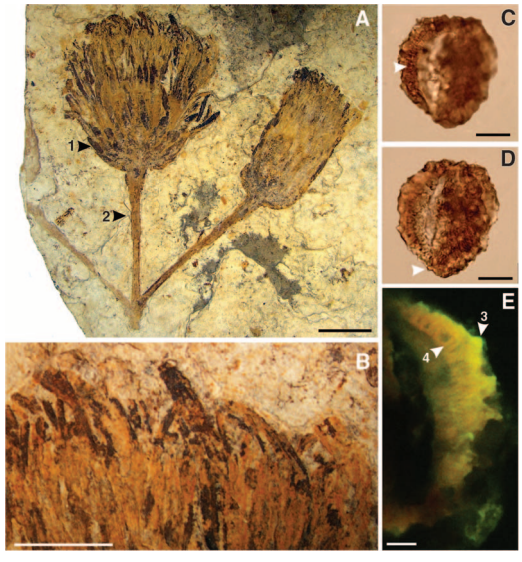


Fig. 1. Fossil Asteraceae. (**A**) Capitulescence under transmitted white light. Label 1 indicates involucral bracts (arrow); 2, peduncle (arrow). (**B**) Flowers. (**C** and **D**) Pollen grains at equatorial view under transmitted white light: (C) exine structure (arrow) and (D) spines arrangement (arrow). (**E**) Optical section of the pollen grain under fluorescent light with confocal microscope; 3, spines (arrow); 4, columellae (arrow). Scale bar in (A) and (B) equals 1 cm; in (C) and (D), 10 μm; and in (E), 5 μm.

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The Ubiquity of Micrometer-Sized Dust Grains in the Dense Interstellar Medium

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Cold molecular clouds are the birthplaces of stars and planets, where dense cores of gas collapse to form protostars. The dust mixed in these clouds is thought to be made of grains of an average size of 0.1 micrometer. We report the widespread detection of the coreshine effect as a direct sign of the existence of grown, micrometer-sized dust grains. This effect is seen in half of the cores we have analyzed in our survey, spanning all Galactic longitudes, and is dominated by changes in the internal properties and local environment of the cores, implying that the coreshine effect can be used to constrain fundamental core properties such as the three-dimensional density structure and ages and also the grain characteristics themselves.

The formation of stars and planets begins in the cold condensed interstellar medium (*1*) through mechanisms that are not yet well understood (*2*). Thus, much attention is currently paid to characterizing the physical conditions of the dense cloud cores where stars form, before and during their collapse (*3*–*5*), up to the time when a circumstellar disk has formed as the host of future planets (*6*). The materials out of which Earth-like planets form are small dust grains embedded in interstellar gas; it has been shown that such grains settle to the disk midplane and grow by coagulation (*7*). In the earlier core phase, coagulation models propose that the dust grains can grow to about micrometer size on time scales that depend on the core's properties (*8*, *9*). Furthermore, extinction and emission measurements of cores have been interpreted to contain evidence for the presence of larger grains (*10*–*13*). In these models, the difficulty is to disentangle the mixed information about density, dust properties, and temperature.

A recent analysis of the nearby cloud L183 using maps produced by the Spitzer Space Telescope Infrared Array Camera (IRAC) (*14*) revealed unexpected emission at 3.6 μm that is spatially coincident with the densest regions of the cloud (*15*). This pattern can only be accounted for if mid-infrared interstellar radiation is scattered by dust grains in the dense cores that are larger than the standard grains decribed in (*15*, *16*). By analogy to the scattered light that has been observed in the outer parts of the cloud at shorter wavelengths and was named "cloudshine" (*17*), the mid-infrared scattered light from the dense cores has been named "coreshine" (*15*). The maximal grain size visible in the coreshine of L183 is about 1 μm and could even be larger in the optically thick center.

We have investigated a sample of 110 cores from the main Spitzer core surveys, without any further selection criterion. This survey includes 12 objects with far-infrared shadow, for which a recent study noticed a 3.6-μm excess emission in 7 to 10 of the studied cores (*5*). In this sample, 95 cores are detectable in the IRAC bands, 50 show clear coreshine, and 8 more cores need further analysis to confirm possible coreshine. Thus, about half of all low-mass cores in our sample show this effect. The fluxes range from 0.01 up to 0.5 MJy/sr, as compared to the background field peak flux at 3.5 μm of about 12 MJy/sr. Some of the most prominent examples of sources with coreshine are displayed in Fig. 1. The dust in the core absorbs the background radiation at 8 μm (righthand image for each core) while it also scatters the external radiation field at 3.6 μm (lefthand image for each core). The good spatial correlation of the scattered light flux at 3.6 μm with the extinction at 8.0 μm shows that the coreshine comes from the dense parts of the molecular cloud cores. These examples include cores with simple geometry such as L1507, binary cores such as L1262, filamentary structures such as L1251A, cores with infall signature such as L1544 (*18*), cores with a protostellar object such as L1521F, or cores with jet activity such as L1157. Thus, coreshine can appear with or without the presence of a class 0 or class I protostar or of a strong outflow.

Aside from scattered light, the emission at mid-infrared wavelengths could also come from stochastically heated small dust grains such as polycyclic aromatic hydrocarbons (PAHs) (*19*). However, an excitation of these grains requires

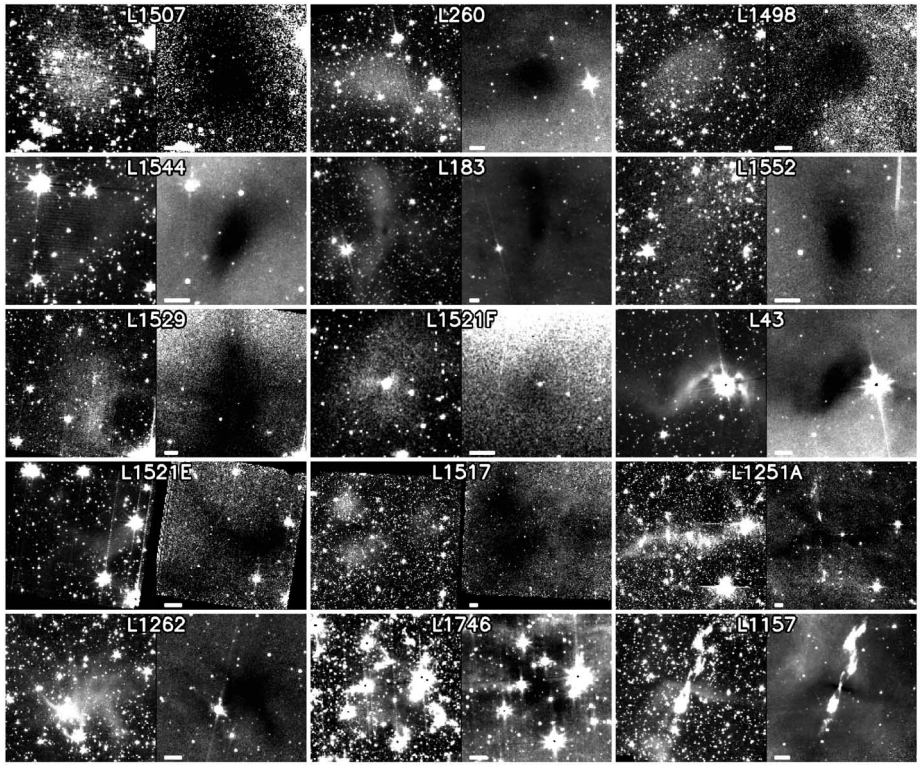


Fig. 1. Fifteen cores with prominent coreshine. For each core, the IRAC 3.6-μm (left) and 8.0-μm (right) Spitzer maps are shown. The white bar in the bottom left corner of the 8-μm images represents a length of 4000 AU.

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exposure to the ultraviolet (UV) and optical interstellar radiation field. This radiation is absorbed and scattered in the outer edges of the clouds, whereas the coreshine comes from the inner dense regions lacking any UV or optical illumination (*15*). The ρ Ophiuchi 9 region (*20*) is an example of a cloud with two cores, where both emission types can be seen in the four IRAC bands observed with the Spitzer telescope (Fig. 2). The PAH emission is visible toward the bright rim on the left, especially at 5.8 and 8 μm, whereas the 3.6-μm coreshine comes from the region seen in extinction at 8 μm around the small core at the lower right. The flux ratio 8:3.6 μm (Fig. 2) reveals the well-correlated coreshine and extinction region.

The coreshine effect is common among different types of cores and is also present across a variety of star-forming environments (Fig. 3). Most nearby star-forming regions contain cores with and without coreshine, which indicates that the appearance of larger grains depends on the local properties and history of the core. Only in the Gum/Vela nebula region has no coreshine

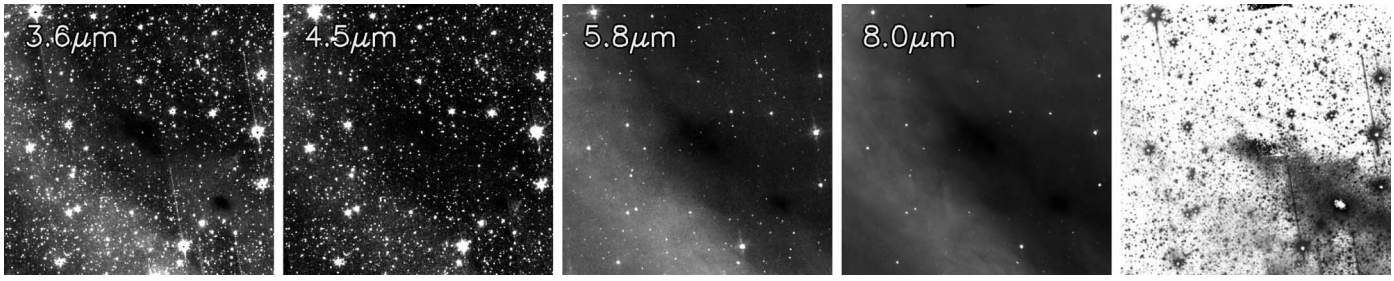


Fig. 2. Coreshine and PAH emission in ρ Ophiuchi 9. (**Left**) The four IRAC Spitzer images at 3.6, 4.5, 5.8, and 8.0 μm. (**Right**) The flux ratio of the images at 8 and 3.6 μm reveals the well-correlated coreshine and extinction region. The size of each frame is 128,000 AU.

Fig. 3. Spatial distribution of cores with and without the coreshine effect. The positions of the investigated cores are plotted over the interstellar radiation all-sky map at 3.5 μm [Diffuse Infrared Background Experiment (*24*)], representing the background field, which is scattered by grains. The center of the Galaxy is at the center of the map, and the anticenter is at the left and right borders. The coreshine flux is indicated as squares scaling logarithmically with the flux. Crosses indicate cores without measurable coreshine flux. Well-known star formation regions are annotated by letters: A, Taurus; B, Cepheus; C, Aquila; D, Ophiuchus; E, Lupus; F, Crux; G, Vela.

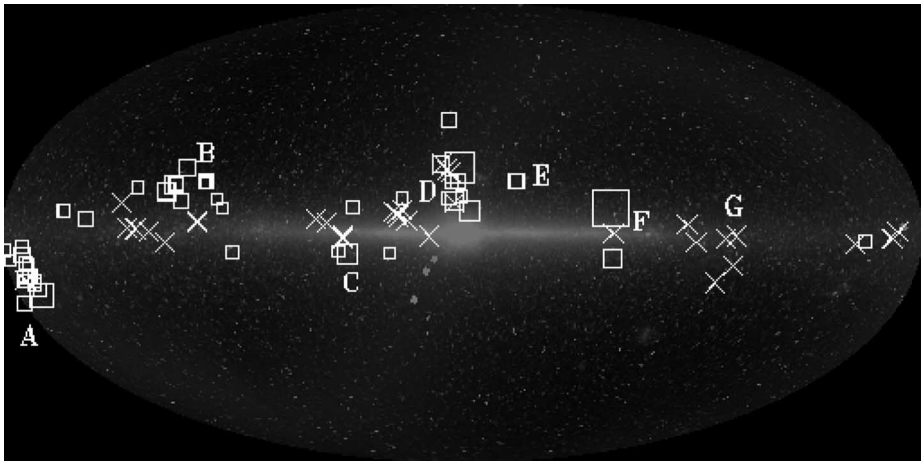
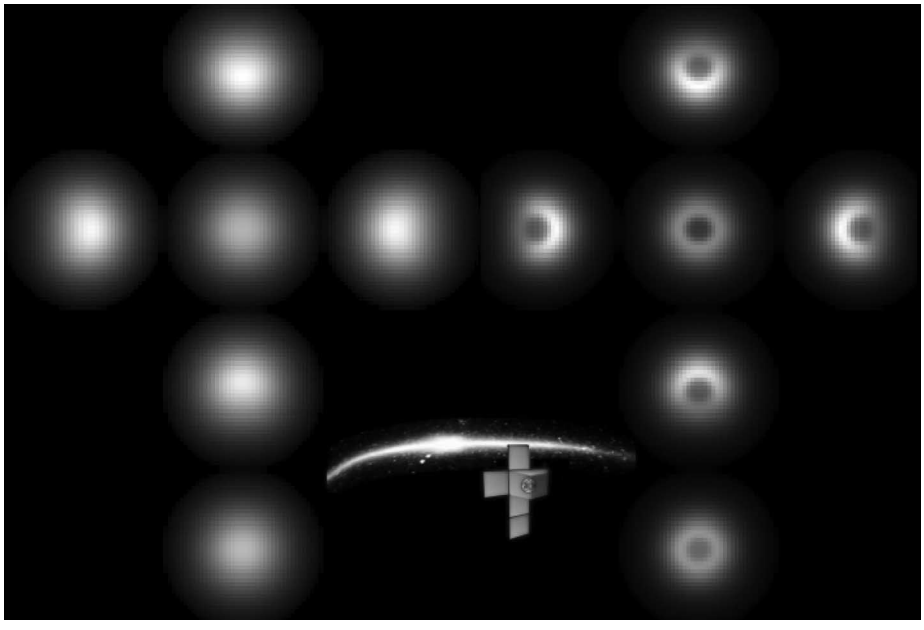


Fig. 4. Coreshine model across the Galaxy. Scattered-light radiative transfer modeling images of the coreshine pattern of a molecular cloud core are shown. The core is placed on a rectilinear coordinate system in which one axis is the line from Earth to the Galactic center (therefore the lowest image is the view to the Galactic anticenter). Coreshine for a 10–solar-mass core of diameter 20,000 AU is shown. (**Left**) With a profile flattening at a radius of 4000 AU. (**Right**) With a flattening at 1000 AU. The box materializes the six directions and their orientation with respect to the Galactic plane (represented by the curved image of the DIRBE 3.6-μm image). The unfolding of the box relates the modeling images to the six directions. The face at the center of the unfolded box indicates the direction aiming at the Galactic center.



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been detected so far. It is possible that a fraction of the large grains in this region might have been destroyed by a supernova that is thought to have reshaped the region about 1 million years ago (*21*). There are not many cases of local cores with detectable coreshine close to the Galactic plane (Fig. 3). It is not presently clear whether this is due to the increased number of background stars confusing the coreshine signal or to physical effects that cause changes in the dust opacities with Galactic position (*22*).

To investigate the influence of the anisotropic illumination on the coreshine appearance with varying Galactic position, we performed three-dimensional (3D) radiative transfer calculations (*23*) for a 10–solar-mass core (Fig. 4). We used a radial density power-law profile in the outer parts (index –1.8) and a flattening toward the center, in agreement with core density profile estimates (*3*). Two different cases were considered, either with a flattening in the innermost 4000 astronomical units [(AU) 1 AU = 1.496 10^{11} m], or with a more centrally concentrated density profile, flattening only in the innermost 1000 AU. The coreshine flux in the core with a large flattening varies little with Galactic core position. However, our model shows a slight increase in flux in the direction toward the Galactic center in the four cases where the core is viewed at 90° from the Galactic center line of sight. More prominently, the case with a more concentrated density profile reproduces the central flux depression that is seen in a few dense cores such as L183 and L1544, where the optical depth at 3.6 μm becomes too large for scattered photons to escape. In the investigated sample, only a handful of cores show this inner flux depression. The asymmetrical flux increase for the four cores that are 90° from the Galactic center line of sight is also more pronounced than in the flatter-profile cases. In both types of profile, for the central image where the core is placed in front of the Galactic center, the photons are scattered in the forward direction but have to cross the core, so that the flux is smaller than for the side-illumination cases. In general, the coreshine effect is therefore only weakly dependent on the location of the source in the Galaxy and is dominated by the properties of the core.

The coreshine effect thus provides a direct tool to investigate the grain and ice-mantle growth process, in which turbulence and density are major ingredients (*9*). Because the growth process is continuous, it can serve as a measure of the age of cores, contrary to chemical "clocks," which may suffer from repetitive resets (*1*). A "grain-growth clock" may help distinguish between the currently proposed models (*1*) of how prestellar cores are stabilized before they collapse.

In the optically thin case, the coreshine flux has the potential to independently provide a measurement of the dust column density. Because scattering is highly sensitive to grain size (*15*), the coreshine effect will help improve our understanding of the grain infrared opacities. Moreover, the combined consideration of scattering, extinction, and emission modeling will constrain the detailed 3D structure of cold molecular clouds down to the cores.

The growth of grains has an impact on the ion-neutral cold chemistry within the core via electronic equilibrium and possibly surface reactions, whereas circumstellar disks might be affected by a seed population already containing large particles, as revealed by the coreshine effect. More generally, the grains unveiled by coreshine might help to characterize the recent history of an entire region, as in the case of the Gum Nebula complex, where an absence of coreshine implies the recent destruction of big dust grains, possibly by a supernova explosion.

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Observing Supernova 1987A with the Refurbished Hubble Space Telescope

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Observations with the Hubble Space Telescope (HST), conducted since 1990, now offer an unprecedented glimpse into fast astrophysical shocks in the young remnant of supernova 1987A. Comparing observations taken in 2010 with the use of the refurbished instruments on HST with data taken in 2004, just before the Space Telescope Imaging Spectrograph failed, we find that the Lyα and Hα lines from shock emission continue to brighten, whereas their maximum velocities continue to decrease. We observe broad, blueshifted Lyα, which we attribute to resonant scattering of photons emitted from hot spots on the equatorial ring. We also detect N v λλ1239, 1243 angstrom line emission, but only to the red of Lyα. The profiles of the N v lines differ markedly from that of Hα, suggesting that the N^{4+} ions are scattered and accelerated by turbulent electromagnetic fields that isotropize the ions in the collisionless shock.

The death of a massive star produces a violent explosion known as a supernova (SN), which expels matter at hypersonic velocities. Supernovae deposit large amounts of mechanical energy and nucleosynthesized elements into the surrounding interstellar medium, driving the physical and chemical evolution of galaxies. The shock impact of the SN debris with ambient matter creates a radiating system known as a supernova remnant. SN 1987A (first observed on 23 February 1987), the brightest such event observed since Kepler's supernova (SN 1604) (*1*), provides a unique opportunity to witness the development of a supernova remnant (*2*, *3*). Because SN 1987A is only 50 kpc away in the Large Magellanic Cloud, the Hubble Space Telescope's (HST's) superb angular resolution is sufficient to re-

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solve the interaction of its shocks with circumstellar material. To track and interpret the temporal, spatial, and spectral evolution of SN1987A, we present observations obtained on 31 January 2010 with the recently repaired Space Telescope Imaging Spectrograph (STIS) and compare them with the results from the last epoch of observations (18 to 23 July 2004) (*4*), before the instrument's failure in August 2004.

The rapidly expanding debris of a SN explosion interacts hydrodynamically with circumstellar matter. If the circumstellar matter has a smooth density distribution, a double-shock structure will be established (*5*). A forward shock (blast wave) propagates into the circumstellar matter, creating a layer of hot, shocked gas. The pressure of this layer drives a reverse shock (RS) into the SN debris. This double-shock structure propagates outward until the blast wave encounters a relatively dense obstacle. In the case of SN 1987A, encountering the equatorial ring drives a reflected shock backward into the debris that merges it with the RS.

The equatorial ring is a relatively dense (atomic hydrogen densities of $n_H \sim 10^3$ to 10^4 atoms cm^{-3}) structure of diameter 1.34 light years (ly), inclined at an angle $i = 45°$ with respect to the line of sight (*6*–*8*). This ring is attributed to a mass-loss event that occurred ~20,000 years before the SN explosion (*9*, *10*). The first evidence of interaction of the blast wave with the ring appeared in 1995, when a rapidly brightening optical "hot spot" appeared in images taken with the Wide Field Planetary Camera 2 aboard the HST (*11*, *12*). Today, the ring is encircled by ~30 hot spots (Fig. 1). Movie S1 shows the emergence of these hot spots and the expansion of the debris from 1994 to 2006. The location of these emission spots just inside the ring and their long duration suggests that they result from shocks that propagate into dense fingers that protrude inward from the equatorial ring (*13*).

Radiation from the RS can be observed at optical and ultraviolet wavelengths. Before it reaches the RS, the outer layer of the SN debris consists mostly of partially ionized hydrogen and helium gas that has been expanding freely since the explosion. When neutral hydrogen atoms cross the RS, they are excited and ionized by collisions with ions in the shocked plasma. If the atoms are excited before they are ionized, they will produce Lyα (1216 Å) and Hα (6563 Å) line emission. On average, about 1 Lyα photon and 0.2 Hα photons are produced for every hydrogen atom crossing the RS (*14*–*16*).

The emission properties of the RS in SN 1987A are similar to those of the Balmer-dominated shock emission observed in several supernova remnants, where photons are produced via collisional excitation (and charge exchange) rather than recombination (*14*, *17*, *18*). In other collisionless supernova remnants, the blast wave overtakes nearly stationary circumstellar matter, whereas in SN 1987A, fast-moving hydrogen gas in the SN debris overtakes the RS. As the hydrogen atoms in the SN debris cross the RS, they freely stream with radial velocity $v_r = r/t_e$, where r is the radius of the RS measured from the explosion center, and t_e is the time since the explosion. Likewise, the atoms have Doppler velocity (projected along the line of sight) $v_z = -r\cos\theta/t_e$, where θ is the angle between the streaming SN debris and the line of sight to the observer. When they are excited by collisions with the shocked gas, the neutral hydrogen atoms are not deflected, so the Doppler shifts of the resulting emission lines that we observe (Fig. 1) correspond to the projected ballistic velocity of the unshocked SN debris.

The unique mapping between distance along the line-of-sight and Doppler shift allows us to

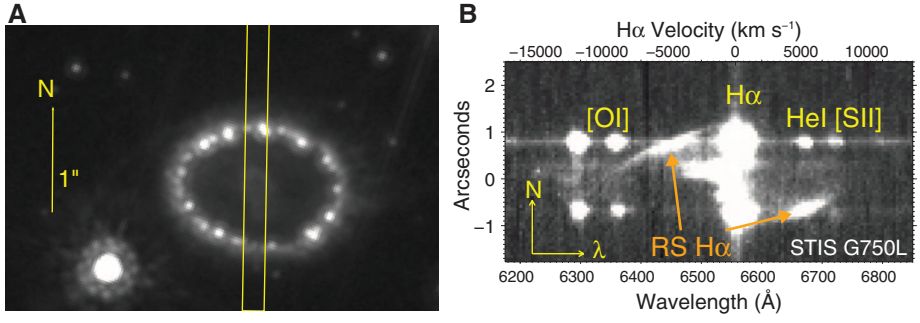


Fig. 1. (**A**) HST-Advanced Camera for Surveys image obtained on 28 November 2003 in the F625W filter with an exposure time of 800 s, illustrating the slit orientation used in our experiment. N, north. (**B**) STIS G750L spectrum of SN 1987A obtained on 31 January 2010, centered on the Hα emission line. The vertical bar at the center of the image is stationary Hα emission from interstellar or circumstellar gas. The bright spots at the north and south of this bar are the emissions from Hα + [N II] λλ6548, 6583 Å from hot spots on the equatorial ring. [O I] λλ6300, 6364, He i λ 6678, and [S II] λλ6716, 6731 Å emission from the hot spots is also observed. The blueshifted streaks near the center are Hα emission excited by radioactivity in the interior of the SN debris. The curved, blueshifted streak extending from the north side of the vertical bar and the redshifted streak on the south side (denoted with orange arrows) are Hα emission from the brightest parts of the RS.

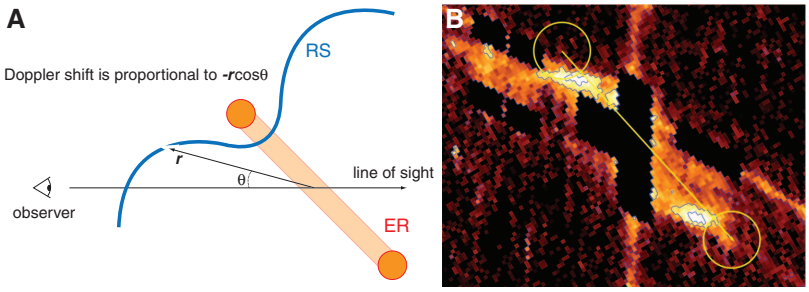


Fig. 2. (**A**) Schematic illustration of the location of the RS with respect to the equatorial ring (ER), which is inclined 45° to the line of sight. (**B**) Hα emission from the RS transformed into a cross-sectional view through the circumstellar ring (see text). The diameter of the equatorial ring is 1.34 ly. The top and bottom yellow circles represent the near (north) and far (south) sides of the equatorial plane of the ring, respectively. The circles represent the cross section of the ring, and contours highlight the emission peaks on the northern and southern streaks.

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convert the spectrum of Fig. 1 into a map of the location of the RS (Fig. 2). The Hα emission is concentrated just inside the equatorial ring, because at these locations, the RS penetrates into denser regions of the SN envelope, where the flow is held back by shocks reflected from the ring. The net Hα flux observed through the 52″ × 0.2″ slit in the total RS (northern blueshifted plus southern redshifted streaks, Fig. 1) is 3.3 (±0.5) × 10^{-13} erg cm^{-2} s^{-1} (*19*). This value is a factor of about 1.7 greater than that measured in July 2004 (*4*) and February 2005 (*20*).

Unlike Hα photons, Lyα photons experience resonant scattering by hydrogen atoms. Thus, Lyα emission is not observed at wavelengths immediately blue- and redward of 1216 Å because of scattering by hydrogen atoms in the Milky Way and Large Magellanic Cloud (Fig. 3). Figure 4 shows comparisons of one-dimensional (1D) scans of Lyα (dark streaks in Fig. 3) and Hα (bright streaks in Fig. 1) emission from the RS. The observed Hα and Lyα fluxes have been increased by factors of ≈ 1.5 and 8, respectively, to correct for interstellar extinction along the line of sight to SN 1987A (*21*, *22*). On the north side of the equatorial ring, the ratio of Lyα to Hα photon fluxes has a fairly constant value near 40 for velocities between –2500 and –6000 km s^{-1} (Fig. 4). This ratio is much greater than the expected photon production ratio of 5:1 for a Balmer-dominated shock (*15*, *16*). Moreover, the Hα emission fades for blueshift velocities < –8000 km s^{-1}, whereas the Lyα emission remains bright for blueshift velocities approaching –12,000 km s^{-1}. If the Lyα photons are produced by the same mechanism as the Hα photons, then the Lyα-to-Hα ratio should be the same for all observed velocities, but it is not. Therefore, most of the observed Lyα emission cannot be produced directly by hydrogen atoms crossing the RS. Unlike Hα, the broad Lyα emission is not confined to a narrow strip delineating the RS surface (Fig. 3).

We propose a different mechanism to account for the highly blueshifted Lyα emission. As the SN blast wave enters the equatorial ring, the shocked hot spots on the ring become bright

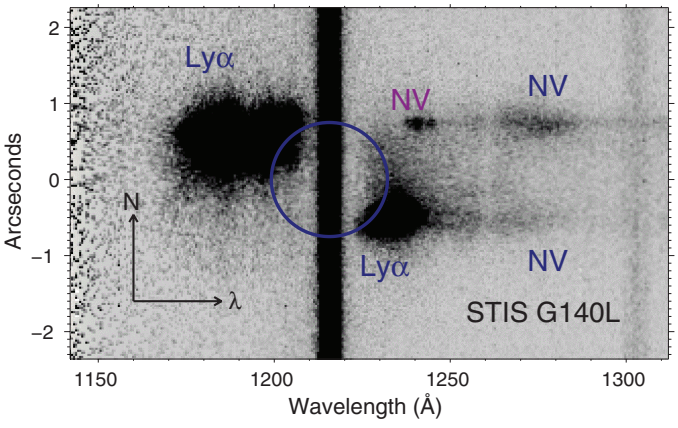


Fig. 3. STIS G140L observations of SN 1987A, acquired on 31 January 2010. The black vertical stripe is the slit image in geocoronal Lyα. The blue circle approximates the location of the circumstellar emission ring. Broad, faint features seen on the north and south sides between about 1260 and 1290 Å (labeled NV in blue) are produced by nitrogen ions in the RS, and the shock-excited N v hot-spot emission is labeled in magenta.

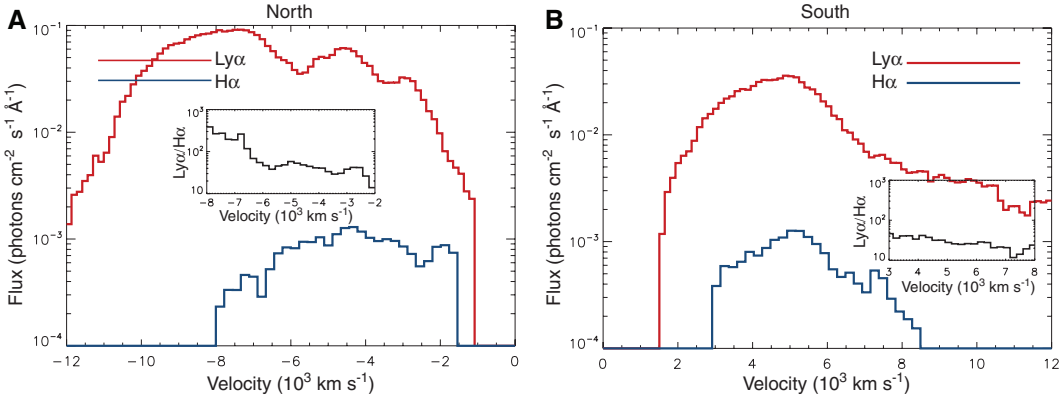


Fig. 4. (**A**) Integrated Hα and Lyα emission from the RS on the north side of the equatorial ring. Although the Hα emission approaches zero blueward of –8000 km s^{-1}, it has been artificially truncated in this plot because of strong contamination from [O I] λλ6300, 6364 Å present in the hot spot (Fig. 1). (Inset) Ratio of Lyα to Hα. The broad dip in the Lyα emission near –6000 km s^{-1} may be due to absorption by interstellar Si ii (λλ1190, 1193 Å). Interstellar absorptions from Si ii (λλ1190, 1193 Å; λλ1260, 1265 Å; and λλ1526,1533 Å) are clearly detected on the sightline to a nearby star. (**B**) Velocity profiles for the southern, redshifted emission. We truncate the southern Hα profile at $v \geq 8500$ km s^{-1} because the Hα flux at these velocities is consistent with a combination of detector background and [S II] emission scattered inside of the equatorial-ring radius.

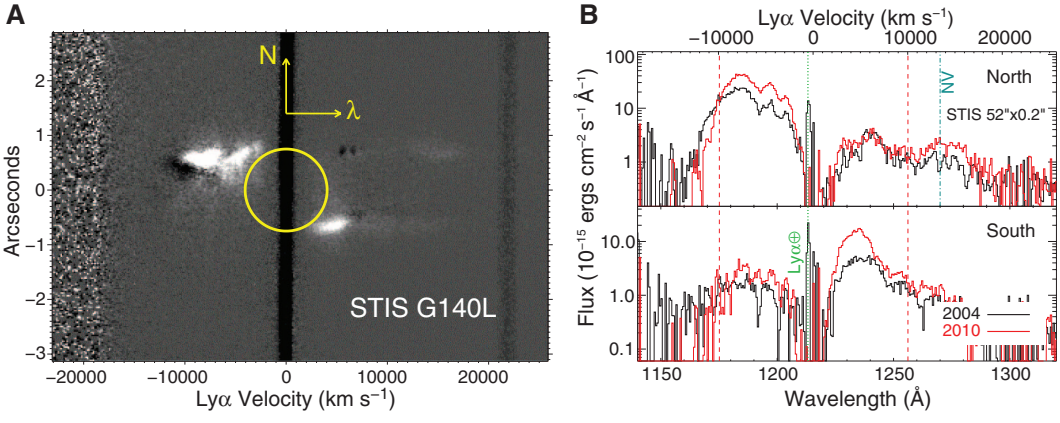


Fig. 5. (**A**) Lyα 2010 to 2004 difference image, scaled such that gray indicates similar intensities in the two epochs. The yellow circle approximates the location of the circumstellar emission ring. (**B**) 1D spectra of the Lyα emission from the north and south regions (*27*). The narrow feature labeled "⊕" is residual emission from Earth's upper atmosphere.

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sources of Lyα radiation. This radiation is invisible to observers on Earth because it is centered at zero velocity with respect to the interstellar neutral hydrogen, and its linewidth is narrow ($\Delta v < 300$ km s^{-1}) (*8*, *13*) so that it is entirely blocked by the interstellar medium. Roughly half of this radiation propagates inward into the SN debris, where the Lyα photons may be resonantly scattered by hydrogen atoms that are expanding with radial velocities ranging from 3000 to 9000 km s^{-1}. In the rest frame of the hydrogen atoms in the expanding debris, photons propagating inward are blueshifted. If they are then scattered backward toward Earth, they will be blueshifted a second time. There is no corresponding bright, high-velocity, redshifted Lyα component on the south side of the equatorial ring (Figs. 3 and 4). The Lyα photons that are emitted radially inward by the ring hot spots on the south side are seen as blueshifted by hydrogen atoms in the onrushing debris. Unlike the case in the north, photons from the south that are scattered toward the observer receive a redshift that cancels out this blueshift. This leaves the Lyα photons at approximately zero velocity, where they will be scattered or absorbed before reaching us.

To check the plausibility of such a mechanism, it is necessary to verify that a sufficient number of Lyα photons are emitted by the hot spots to account for the observed high-velocity Lyα and that the neutral hydrogen layer in the expanding SN envelope has sufficient optical depth to scatter Lyα photons by roughly half of the observed maximum velocity (i.e., 6000 km s^{-1}). It is not possible to measure the emitted Lyα flux from the hot spots directly because this radiation is blocked by interstellar hydrogen atoms. We can estimate the strength of Lyα emission using the direct Hα emission from the ring, applying a theoretical scaling [Lyα:Hα ~ 5 to 10; (*23*)]. The Hα hot-spot emission within the STIS slit in the 2010 observations ($\approx 2.1 \times 10^{-12}$ erg cm^{-2} s^{-1}) suggests that the hot-spot Lyα flux in 2010 is ~3 to 7 photons cm^{-2} s^{-1}. This is sufficient to account for the broad blueshifted Lyα emission (Fig. 4).

Regarding the question of optical depth in the line wings of Lyα, the radial column density of hydrogen atoms in the debris is roughly $N_H \approx M_H/(4\pi R^2 m_H)$, where $M_H \sim 5M_\odot$ (here, $M_\odot$ is the mass of the Sun) is the estimated mass of hydrogen in the SN envelope, $R \approx 6 \times 10^{17}$ cm is the radius of the envelope, and m_H is the mass of the hydrogen atom. For these estimated values, $N_H \approx 1.3 \times 10^{21}$ cm^{-2}. The optical depth of such a column to Lyα photons, Doppler shifted by $v_{1000} = v/(1000$ km s$^{-1})$ (where v is the velocity), is given roughly by $\tau \approx N_H \sigma_o \{(\gamma/4\pi^2)/[\Delta f^2 + (\gamma/4\pi)^2]\}$ (*24*), where the frequency shift is $\Delta f/f_0 = v/c$ (here, c is the speed of light), σ_o is the line-center absorption cross section, and $\gamma = 6.3 \times 10^8$ s^{-1} is the spontaneous decay rate of electrons in the excited state of the hydrogen atom leading to the emission of Lyα photons. For $v_{1000} = -6$, $\tau \approx 0.1$; for comparison, $\tau > 2.5$ for $|v_{1000}| < 1$. Our estimate of the available Lyα photon budget from the hot spots provides an ample margin for this mechanism to operate, even if <50% of the hot-spot Lyα enters the debris. A measurable fraction of the Lyα photons emitted by a hot spot that enter the SN debris will be backscattered and emerge with blueshifts ranging up to ~ –12,000 km s^{-1}.

The Lyα emission brightened from 2004 to 2010 (Fig. 5). We observed two primary features: (i) The Lyα emission has increased in brightness by factors of 1.6 to 2.4 for the north and south shock emission, and (ii) the maximum Doppler shift in the northern blueshifted Lyα emission is decreasing as a function of time. We also note the faint glow seen on the north and south sides at wavelengths ranging from about 1260 to 1290 Å, also visible in Fig. 3. This emission cannot be attributed to Lyα, because it would require the Lyα emission on the north side to be redshifted by velocities up to +20,000 km/s, whereas the actual Lyα emission on that side is blueshifted (Fig. 3).

We propose that this emission comes from fast-moving N^{4+} ions in a thin layer immediately downstream from the RS. Neutral or singly ionized nitrogen atoms that cross the RS and enter the shocked plasma are repeatedly ionized by collisions in the shock transition zone. As it passes through the Li-like ionization stage (N^{4+}), a nitrogen atom may be excited to the 2^2P fine-structure state and emit a N v 1239 or 1243 Å photon, or it may be ionized to N^{5+}. The number of N v photons produced per nitrogen atom passing through the RS will be equal to the ratio of the N^{4+} 2^2P excitation rate to its ionization rate. The Lyα excitation rate is approximately equal to the ionization rate; however, the excitation rate producing the N v emission (which is dominated by collisions with protons and alpha particles) exceeds the ionization rate by a factor of several hundred (*25*, *26*). We estimate that each nitrogen atom that passes through the RS will emit ~600 N v photons before it becomes fully ionized. Given the enriched abundance of nitrogen (N/H ~ 2×10^{-4}) (*7*) in the equatorial ring (and presumably in the outer debris of SN 1987A), the identification of these emissions as N v is plausible.

The putative N v emission is redshifted on the northern side of the RS, because the nitrogen atoms are ionized and accelerated by the turbulent electromagnetic fields in the isotropization zone of the collisionless shock before they emit N v photons. This is in contrast to the hydrogen atoms, which are not deflected notably from free expansion when they emit Lyα. If our identification of this faint feature as N v is correct, we are seeing redshifts on the north side extending to ~12,000 km s^{-1}. The profile of the N v emission will be a convolution of the projected velocity distribution function of the N^{4+} ions with the shock surface, both of which are unknown. But it is probable that the line profile will also have a blueshifted wing extending to at least ~ –12,000 km s^{-1}. We cannot discern this wing because it will be buried under the much brighter Lyα emission. A critical test of our hypothesis will be the observation of the profile of the C iv λλ1548, 1550 Å doublet, which we estimate to have a brightness ~0.3 times that of N v λλ1239, 1243 Å. The C iv doublet should have the same intrinsic emission profile as N v, but it will not be confused with Lyα emission and absorption.

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Measurement of Fast Electron Spin Relaxation Times with Atomic Resolution

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Single spins in solid-state systems are often considered prime candidates for the storage of quantum information, and their interaction with the environment the main limiting factor for the realization of such schemes. The lifetime of an excited spin state is a sensitive measure of this interaction, but extending the spatial resolution of spin relaxation measurements to the atomic scale has been a challenge. We show how a scanning tunneling microscope can measure electron spin relaxation times of individual atoms adsorbed on a surface using an all-electronic pump-probe measurement scheme. The spin relaxation times of individual Fe-Cu dimers were found to vary between 50 and 250 nanoseconds. Our method can in principle be generalized to monitor the temporal evolution of other dynamical systems.

When a magnetic atom is placed in a solid or onto a surface, it can exchange energy and angular momentum with the local environment, giving a finite lifetime to its excited spin states. For non-itinerant (localized) electron systems, these interactions occur on the atomic length scale and on a time scale generally in the pico- to microsecond range. Magnetic resonance techniques are widely used to measure spin relaxation times (*1*), but achieving high spatial resolution of individual spins rather than ensembles has remained a challenge (*2*–*4*). Spin-polarized scanning tunneling microscopy (STM), by contrast, can probe static magnetic properties on the atomic scale, such as complex magnetic order (*5*), *g*-values (*6*), anisotropy (*7*, *8*) and exchange energies (*9*, *10*). However, the bandwidth of conventional STM current amplifiers (*11*) is insufficient to directly access typical spin relaxation times. Efforts to enhance the STM's temporal resolution through faster current amplifiers (*12*) or pulsed laser techniques (*13*, *14*) have so far lacked atomic spatial resolution.

Here we show that the combination of an all-electronic pump-probe scheme with a spin-sensitive contrast mechanism (*15*, *16*) allows the STM to measure electron spin relaxation times of individual atoms with nanosecond time resolution. In this pump-probe scheme (Fig. 1), we apply a strong voltage pulse (the pump pulse) across the tunnel junction to create spin excitations of the surface atom and a weaker voltage pulse (the probe pulse) to interrogate the state of the atom's spin at a time Δt after the pump pulse (*17*). Integrating the probe-pulse current over many pump-probe cycles and slowly varying Δt allows us to map out the average dynamical evolution of the atom's spin.

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Atomic-scale structures having long spin relaxation times may have applications in information storage or quantum information processing (*18*). It was shown that large easy-axis magnetic anisotropy can lead to long spin relaxation times (*19*) that are sufficient for coherent spin manipulation (*20*). Fe atoms placed on a Cu_2N overlayer on a Cu(100) surface have shown considerable magneto-crystalline anisotropy (*8*). Cu_2N binds the Fe atoms in a polar-covalent network that enables large anisotropy and decouples the adatoms from the Cu conduction electrons (*10*, *21*, *22*), allowing Fe to retain its free atom spin of $S = 2$ (*8*). Here we increased the easy-axis anisotropy of an Fe atom by placing it adjacent to a Cu adatom (Fig. 2A). We probed the dimer using spin-excitation spectroscopy (Fig. 2B) and found the first excitation at a threshold voltage of $V_{thr} = 16.7$ mV, a factor of 4 higher than for the individual Fe atom. This indicates an unusually large easy-axis anisotropy, and indeed we find spin relaxation times that exceed 200 ns.

Spin excitations of the surface atom are driven by inelastic scattering of the tunneling electrons (*6*). In this process, the pump voltage must exceed the threshold voltage, V_{thr}, required to drive the atom's spin from the ground state to an excited state. In general, the magnetic orientation of an excited spin state will be different from that of the ground state. We can sense the orientation of the surface atom's spin by magneto-resistive tunneling with a spin-polarized tip (Fig. 1) (*17*). The tunnel current that flows during the probe pulse then depends on the projection of the surface atom's spin along the spin-polarization axis of the tip.

A pump-probe measurement of an Fe-Cu dimer at 0.6 K temperature and 7 T magnetic field is shown in Fig. 2C. In our experiments, we record the probe-pulse current, which can be expressed as the number of electrons per probe pulse, N. We plot $\Delta N = N(\Delta t) - N(-600\ \text{ns})$, the change in N with respect to its value when the probe pulse precedes the pump pulse, a condition in which the spin is in its ground state during the probe pulse. The behavior of ΔN for positive delay times reveals the dynamical evolution of the Fe-Cu dimer after the end of the pump pulse. Between pump and probe pulse, no voltage was applied that could disturb the free evolution of the surface atom's spin. We observe ΔN to be negative and to exponentially decay to zero as Δt is increased. ΔN is expected to be negative: In the ground state, the surface spin is aligned nearly parallel to the spin-polarization axis of the tip. Any spin excitation reduces the projection of the surface spin along the polarization axis of the tip and hence reduces the conductance of the tunnel junction. The exponential decay of ΔN is characteristic for a system with a single rate-limiting relaxation mechanism. Fitting an exponential to the time dependence of ΔN yields a relaxation time of $T_1 = 87 \pm 1$ ns.

To demonstrate that the observed exponential decay is indeed due to spin relaxation, we performed two control experiments. First, we repeated the measurement on the same Fe-Cu dimer but with a non–spin-polarized tip (Fig. 2C). We switched between spin-polarized and non–spin-polarized STM tips by means of the reversible transfer of a Mn atom from the surface to the apex of the tip (*15*). Without spin polarization of the tip, the conductance of the tunnel junction is insensitive to the orientation of the dimer spin, and we correspondingly observe ΔN to be zero. Second, we repeated the measurement with a spin-polarized tip but on a Cu adatom, which has no features in its spin excitation spectrum (Fig. 2B), consistent with having no net spin. As expected, we observe ΔN to be zero in this case (Fig. 2C).

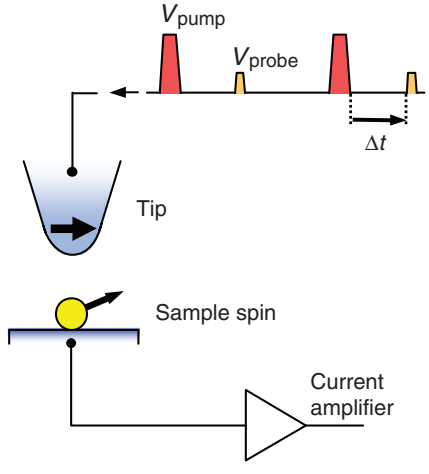


Fig. 1. Spin-sensitive pump-probe measurement scheme. The conductance of the STM tunnel junction varies according to the alignment of the sample spin with the tip spin. A series of fast voltage pulses with voltage V_{pump} for the pump pulse and V_{probe} for the probe pulse is sent to the tip. Measurement of the time-averaged probe-pulse current as a function of the delay time between the pulses, Δt, reveals the dynamical evolution of the sample spin.

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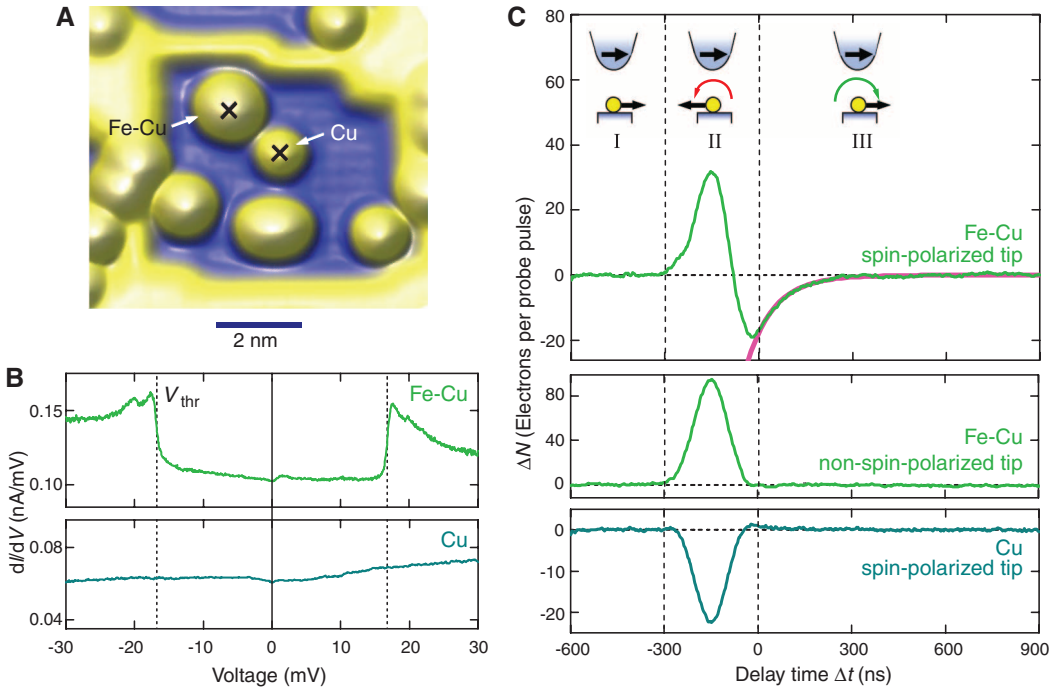


Fig. 2. Measurement of spin relaxation time. (**A**) STM topograph of an Fe-Cu dimer and a Cu adatom (yellow: high; blue: low). (**B**) Spin excitation spectra d*I*/d*V* versus voltage, of an Fe-Cu dimer and a Cu adatom measured with a non–spin-polarized tip at magnetic field $B = 7$ T. Steps in d*I*/d*V* indicate spin excitation energies. (**C**) Pump-probe measurements, ΔN versus Δt, at $B = 7$ T. Region I: probe pulse precedes the pump pulse; region II: pump and probe pulses overlap (fig. S1) (*17*); region III: probe pulse follows the pump pulse and senses the postexcitation dynamics of the system. Insets depict the relative orientation of tip and sample spins. For the Fe-Cu dimer (top panel) ΔN decays exponentially in region III, with a spin relaxation time of $T_1 = 87 \pm 1$ ns obtained from an exponential fit (magenta). Control experiments on the same Fe-Cu dimer but without spin sensitivity in the tip (middle panel) and on a Cu atom with spin-sensitivity in the tip (bottom panel).

We tested whether the pump-probe measurement was itself affecting the spin relaxation time and found that T_1 does not change under variation of the amplitude and widths of the pump and probe pulses and the tip-sample separation (Fig. 3B and fig. S2). However, the magnitude of the pump-probe signal, ΔN, depends strongly on the pump voltage, with a distinct onset at V_{thr}, below which the pump pulse does not lead to a detectable spin relaxation signal (Fig. 3A). This observation agrees with the spin excitation spectrum, where the first spin excitation is observed at the same threshold voltage (Fig. 2B). The polarity of the pump pulse is not crucial, but negative voltages help in exciting the Fe-Cu dimer's spin by spin-momentum transfer from the spin-polarized tip (*15*).

The pump-probe measurements corroborate the finding of large easy-axis magnetic anisotropy for the Fe-Cu dimers. Such anisotropy gives rise to a spin state distribution with two low-lying states, $|+2\rangle$ and $|-2\rangle$, that are separated by an energy barrier (Fig. 3C). In a magnetic field along the easy axis, $|+2\rangle$ is the ground state and $|-2\rangle$ a long-lived excited state. Excitation of the Fe-Cu dimer over the barrier starts with a transition from $|+2\rangle$ to $|+1\rangle$ and requires at least eV_{thr} energy (Fig. 3C, red arrow), which results in the threshold behavior found in the pump-probe measurement (Fig. 3A). Once excited to the $|+1\rangle$ state, successive excitations and de-excitations (Fig. 3C, light red arrows) are immediately possible as none require energy exceeding eV_{thr}. Upon termination of the pump pulse, the spin can be in any of the states but relaxes to either $|+2\rangle$ or $|-2\rangle$ on a time scale that is as yet too rapid for us to observe (*15*). The time-dependent signal recorded in the pump-probe measurement for $t > 0$ (Fig. 2C, region III) then corresponds to the relaxation from the long-lived $|-2\rangle$ state (Fig. 3C, green arrow). At 0.6 K the thermal energy is insufficient to relax over the energy barrier, and indeed no thermally activated relaxation is observed for temperatures between 0.6 and 10 K (fig. S3). Hence, the relaxation out of $|-2\rangle$ is likely due to magnetic tunneling (*23*, *24*), as we discuss next.

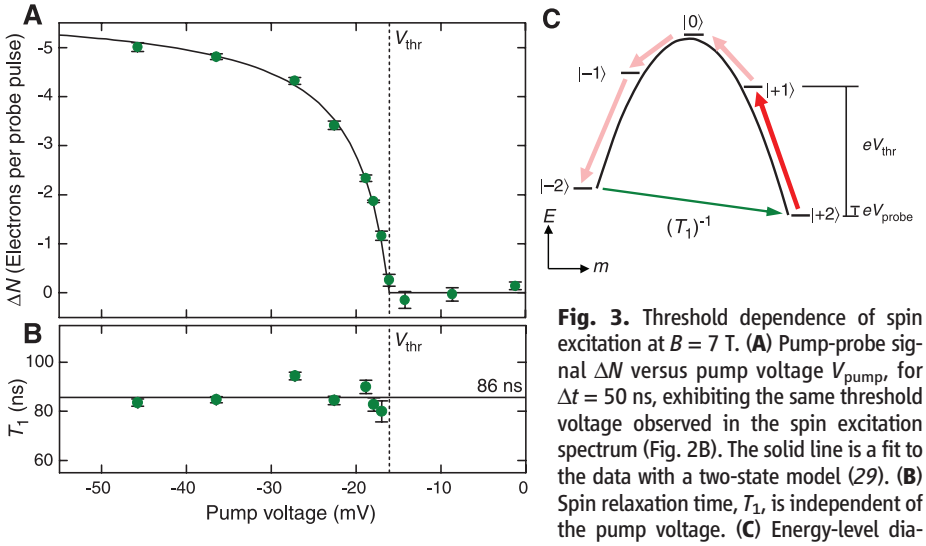


Fig. 3. Threshold dependence of spin excitation at $B = 7$ T. (**A**) Pump-probe signal ΔN versus pump voltage V_{pump}, for $\Delta t = 50$ ns, exhibiting the same threshold voltage observed in the spin excitation spectrum (Fig. 2B). The solid line is a fit to the data with a two-state model (*29*). (**B**) Spin relaxation time, T_1, is independent of the pump voltage. (**C**) Energy-level diagram of the spin states of the Fe-Cu dimer as a function of the magnetic quantum number, m. The measured relaxation time, T_1, corresponds to the $|m = -2\rangle \rightarrow |+2\rangle$ transition.

Figure 4A shows that ΔN decays as a single exponential for all values of magnetic field, but the relaxation time varies strongly with magnetic field. T_1 first grows with magnetic field, but declines rapidly above ~6 T (Fig. 4B). A similar non-monotonous field dependence of T_1 was recently observed for quantum tunneling of magnetization in molecular magnets (*19*). Finite transverse anisotropy makes magnetic tunneling possible for Fe-Cu dimers by mixing the $|-2\rangle$ and $|+2\rangle$ states. As the magnetic field is increased from zero, the Zeeman energy increasingly splits these states, which reduces the tunneling matrix element between them and thereby increases T_1 (*23*). The reduction in T_1 above 6 T indicates a nonzero angle between the magnetic field and the easy magnetic axis of the Fe-Cu dimer. A transverse component of the magnetic field increases mixing of the spin states and shortens T_1 (*25*).

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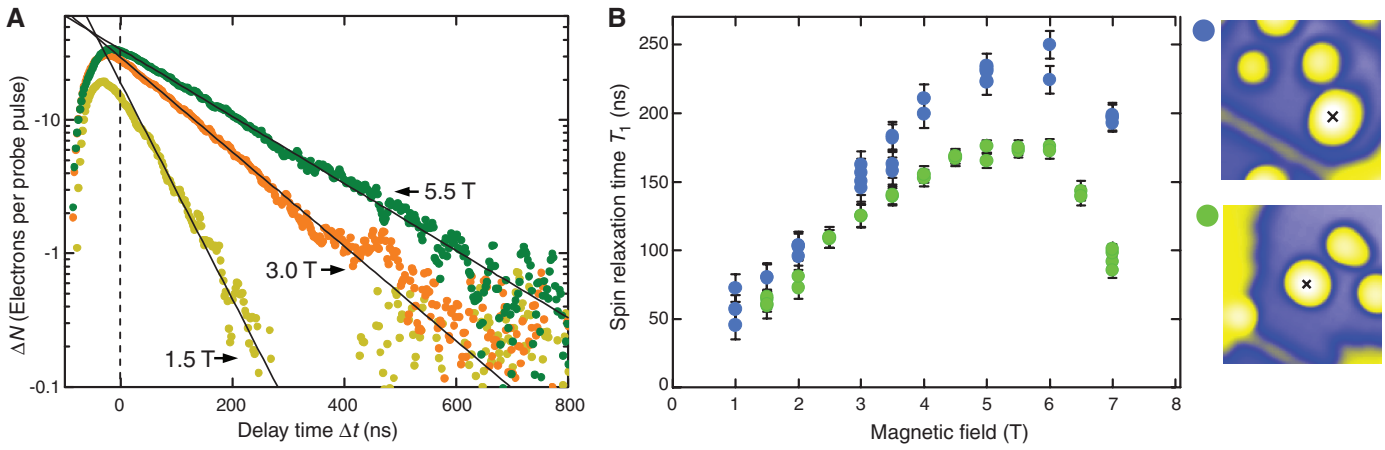


Fig. 4. Field and site dependence of the spin relaxation time. (**A**) Pump-probe measurements for different magnetic fields on an Fe-Cu dimer; solid lines are exponential fits. (**B**) T_1 as a function of magnetic field for the two Fe-Cu dimers shown in the accompanying 5-nm by 5-nm STM topographs.

One of the Fe-Cu dimers in Fig. 4B always exhibits a larger T_1 than the other. We speculate that this variation is due to differences in the nearby surface features as seen in the accompanying topographs. This observation emphasizes the capability of the all-electronic pump-probe technique presented here to resolve local variations in the spin relaxation time with atomic precision.

The pump-probe scheme we have described can be used to monitor the temporal evolution of any excitation provided (i) the excitation can be driven by tunneling electrons; (ii) the conductance of the tunnel junction exhibits a postexcitation time dependence; and (iii) the system evolves on an accessible time scale. Excitations fulfilling these requirements include long-lived vibrational excitations, conformational changes of molecules (*26*) such as in molecular motors (*27*), or fast localized heating (*28*). We emphasize that this pump-probe scheme can in principle be used to monitor the dynamical evolution of the excited state, not just its relaxation; with sufficient temporal resolution it should be possible to monitor the vibration of an atom or molecule and even the precession of a spin.

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Materials and Methods
Figs. S1 to S3
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Movie S1

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Optical Clocks and Relativity

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Observers in relative motion or at different gravitational potentials measure disparate clock rates. These predictions of relativity have previously been observed with atomic clocks at high velocities and with large changes in elevation. We observed time dilation from relative speeds of less than 10 meters per second by comparing two optical atomic clocks connected by a 75-meter length of optical fiber. We can now also detect time dilation due to a change in height near Earth's surface of less than 1 meter. This technique may be extended to the field of geodesy, with applications in geophysics and hydrology as well as in space-based tests of fundamental physics.

Albert Einstein's theory of relativity forced us to alter our concepts of reality. One of the more startling outcomes of the theory is that we have to give up our notions of simultaneity. This is manifest in the so-called twin paradox (*1*), in which a twin sibling who travels on a fast-moving rocket ship returns home younger than the other twin. This "time dilation" can be quantified by comparing the tick rates of identical clocks that accompany the traveler and the stationary observer. Another consequence of Einstein's theory is that clocks run more slowly near massive objects. In the range of speeds and length scales encountered in our daily life, relativistic effects are extremely small. For example, if two identical clocks are separated vertically by 1 km near the surface of Earth, the higher clock emits about three more second-ticks than the lower one in a million years. These effects of relativistic time dilation have been verified in several important experiments (*2*–*6*)

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and are accounted for routinely in satellite-based navigation systems (*7*). The most accurate determinations historically involve velocities near the speed of light (*8*) and changes in elevation of 10^4 to 10^7 m (*3*, *4*). Previously, small relativistic shifts ($<10^{-16}$) could be observed only over short distances in γ-ray Mössbauer spectroscopy (*5*, *9*) and atom interferometry (*6*). However, they might be detected over longer distances by clocks with sufficiently high sensitivity, such as accurate atomic clocks operating in the optical regime, or "optical clocks." Here we report the detection of relativistic time dilation due to velocities of several meters per second and, separately, due to a change in height of 0.33 m by comparing two optical clocks based on $^{27}Al^+$ ions.

With operating frequencies in the petahertz (1 PHz = 10^{15} Hz) range and natural linewidths at the millihertz level, optical clocks have demonstrated substantial improvements in stability and accuracy over the current microwave time and frequency standards (*10*). We compared two optical atomic clocks based on individual trapped Al^+ ions, with reported systematic frequency uncertainties of 8.6×10^{-18} (*11*) and 2.3×10^{-17} (*12*). For comparison, the lowest reported frequency uncertainty of Cs fountain clocks is 3.4×10^{-16} (*13*). With the accuracy and the associated sensitivity of these Al^+ optical clocks, frequency variations below 10^{-16} in the clocks due to relativistic effects can be observed.

Due to the lack of an accessible optical transition in $^{27}Al^+$ for efficient laser cooling and state detection, precision spectroscopy of these ions uses techniques developed in quantum information science. Here, an Al^+ ion is sympathetically cooled through its Coulomb interaction with an auxiliary "logic" ion that is simultaneously held in the same linear radio-frequency (RF) Paul trap (*14*). The logic ion also helps prepare and detect the internal state of the Al^+ ion via quantum logic protocols. In this work, the two Al^+ clocks used a beryllium ($^9Be^+$) ion (*14*) and a magnesium ($^{25}Mg^+$) ion (*11*), respectively, as the logic ion. The Al^+ $^1S_0 \leftrightarrow {}^3P_0$ clock transition with frequency f_0 near 1.121 PHz has a narrow (Δf = 8 mHz) natural linewidth and a corresponding intrinsic quality (Q) factor of $f_0/\Delta f = 1.4 \times 10^{17}$ that permits high sensitivity for detecting small frequency-shifting effects. However, the observed linewidth for the clock transition is limited by the linewidth of the probe laser. We probed the clock transition with a subhertz linewidth laser referenced to a high-finesse optical cavity (*15*). In the Al-Mg clock, with 300 ms probe duration we obtained a narrow, Fourier transform–limited linewidth, realizing a Q factor of 4.2×10^{14} with nearly 80% contrast (Fig. 1). This high-Q line provides the basis for high-stability clock operation and sensitivity to small frequency shifts.

The two Al^+ optical clocks were located in separate laboratories and were compared by transmitting the stable clock signal through a 75-m length of phase-stabilized optical fiber. To observe time dilation due to motion, we set the Al^+ ion in the Al-Mg clock in motion by applying a small static electric field that shifts the position of the ion slightly away from the null of the confining RF field (*16*). The ion is thereby subject to an RF electric field at f_{RF} = 59 MHz (Fig. 2) and undergoes harmonic motion. The ion velocity is adjusted by varying the applied static electric field. In the language of the twin paradox, the moving Al^+ ion is the traveling twin, and its harmonic motion amounts to many round trips. Time dila-

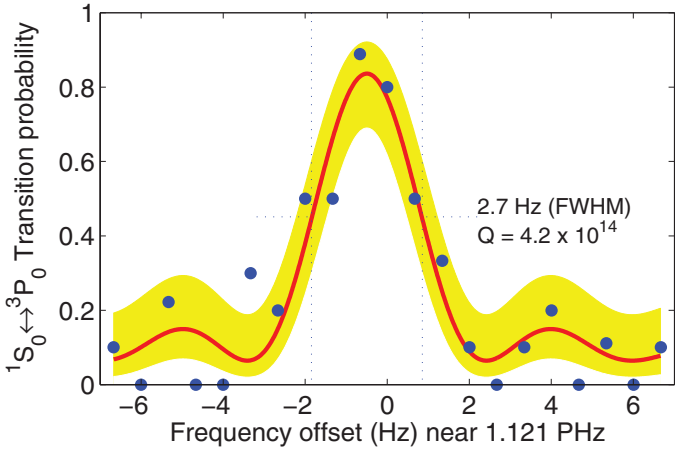


Fig. 1. The $^1S_0 \leftrightarrow {}^3P_0$ transition in Al^+ observed with probe pulses of 300 ms duration. Each datum (blue dots) consists of an average of 9 or 10 individual probes, and the sequence of probe frequencies was randomized to eliminate skewing effects due to frequency drifts in the probe laser. A Lorentzian fit to this data (not shown in the figure) produces a linewidth [full width at half maximum (FWHM)] of 2.30 ± 0.51 Hz (uncertainties correspond to 1 SD). The Lorentzian linewidth is narrower than the Fourier transform–limited spectrum (smooth fitted red curve) that is the result of the rectangular temporal waveform of the probe pulses. For the probe time used here, the Fourier limit corresponds to a linewidth (FWHM) of 2.7 Hz and a Q factor of 4.2×10^{14}, which we take as the achieved values. The yellow shaded band indicates the expected scatter of the data due to quantum projection noise (*34*).

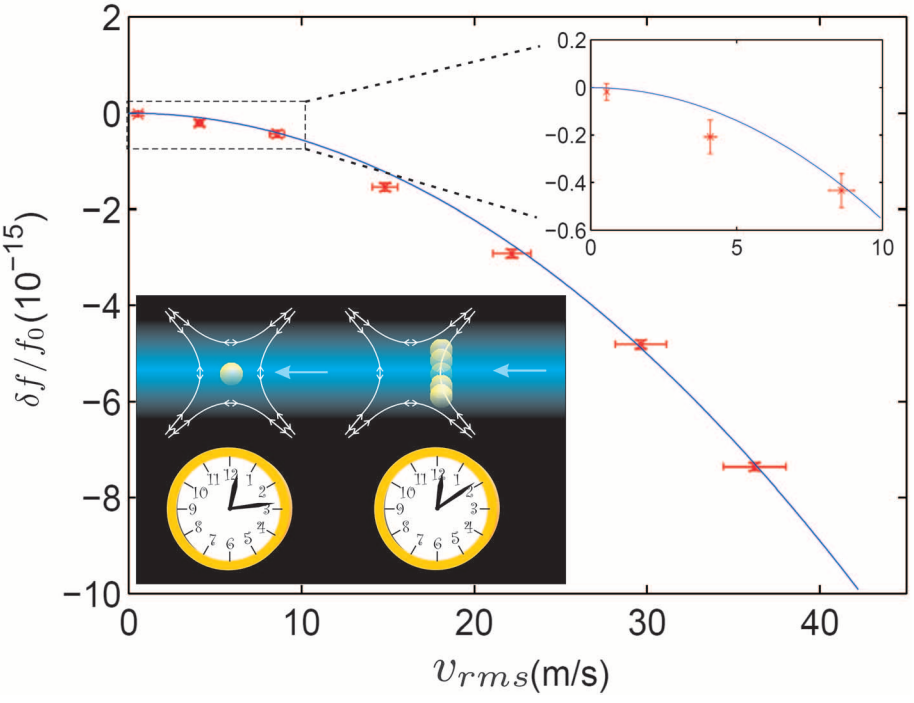
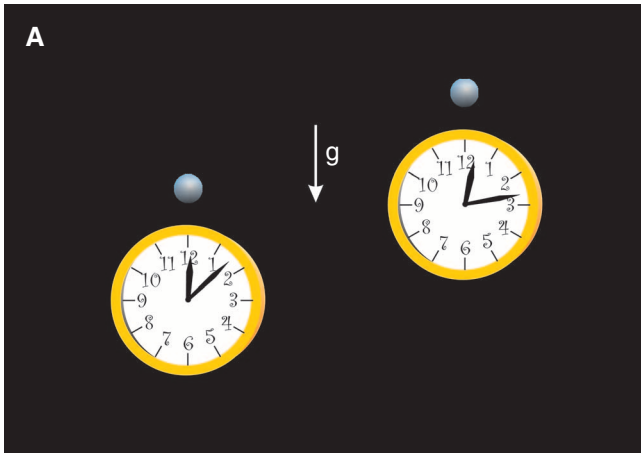


Fig. 2. Relativistic time dilation at familiar speeds (10 m/s = 36 km/hour ≈ 22.4 miles/hour). (Lower left inset) As the Al^+ ion in one of the twin clocks is displaced from the null of the confining RF quadrupole field (white field lines), it undergoes harmonic motion and experiences relativistic time dilation. In the experiments, the motion is approximately perpendicular to the probe laser beam (indicated by the blue shading). The Al^+ ion clock in motion advances at a rate that is slower than its rate at rest. In the figure, the fractional frequency difference between the moving clock and the stationary clock is plotted versus the velocity ($v_{rms} = \sqrt{\langle v^2 \rangle}$) (rms, root mean square) of the moving clock. The solid curve represents the theoretical prediction. (Upper right inset) A close-up of the results for $v_{rms} < 10$ m/s in the dashed box. The vertical error bars represent statistical uncertainties, and the horizontal ones cover the spread of measured velocities at the applied electric fields.

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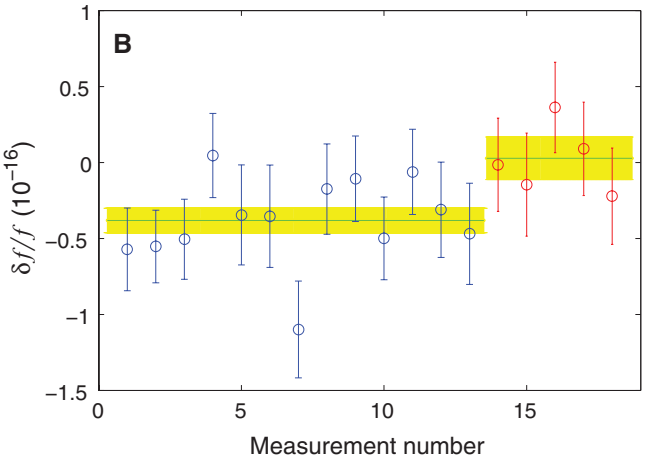


Fig. 3. Gravitational time dilation at the scale of daily life. (**A**) As one of the clocks is raised, its rate increases when compared to the clock rate at deeper gravitational potential. (**B**) The fractional difference in frequency between two Al^+ optical clocks at different heights. The Al-Mg clock was initially 17 cm lower in height than the Al-Be clock, and subsequently, starting at data point 14, elevated by 33 cm. The net relative shift due to the increase in height is measured to be $(4.1 \pm 1.6) \times 10^{-17}$. The vertical error bars represent statistical uncertainties (reduced $\chi^2 = 0.87$). Green lines and yellow shaded bands indicate, respectively, the averages and statistical uncertainties for the first 13 data points (blue symbols) and the remaining 5 data points (red symbols). Each data point represents about 8000 s of clock-comparison data.

tion from this motion leads to a fractional frequency shift for the moving clock of (*17*)

$$\frac{\delta f}{f_0} = \frac{1}{\langle \gamma(1 - v_\parallel/c)\rangle} - 1 \qquad (1)$$

Here $v_\parallel$ is the velocity of the Al^+ ion along the wave vector of the probe laser beam $\gamma = 1/\sqrt{1 - v^2/c^2}$, c is the speed of light, v is the ion's velocity with respect to the laboratory reference frame, and f_0 is the ion's proper resonant frequency. Angle brackets denote time averages. Because the induced Al^+ ion motion is harmonic, its contribution to $\langle v_\parallel \rangle$ averages to zero; therefore, any observed change in the ion's transition frequency is due to a change in γ and corresponds to relativistic time dilation (*18*). For $v/c << 1$, Eq. 1 can be approximated by $\delta f/f_0 \approx -\langle v^2\rangle/2c^2$ (*17*). We measured the frequency difference between the two clocks ($\delta f/f_0$) while varying the velocity of the ion motion. The experimental results, which confirm the prediction of Eq. 1, are plotted in Fig. 2.

Differences in gravitational potential can be detected by comparing the tick rate of two clocks. For small height changes on the surface of Earth, a clock that is higher by a distance Δh runs faster by

$$\frac{\delta f}{f_0} = \frac{g\Delta h}{c^2} \qquad (2)$$

where $g \approx 9.80$ m/s^2 is the local acceleration due to gravity (*4*). The gravitational shift corresponds to a clock shift of about 1.1×10^{-16} per meter of change in height. To observe this shift, we first compared the frequencies of the two Al^+ clocks at the original height difference of $\Delta h = h$(Mg-Al) − h(Be-Al) = −17 cm, which was measured with a laser level. Then we elevated the optical table on which the Mg-Al clock was mounted, supporting it on platforms that increased the height by 33 cm, and compared the frequencies again. The two measurements consist of approximately 100,000 s of low-height data and 40,000 s of high-height data, and the clocks exhibit (Fig. 3) a fractional frequency change of $(4.1 \pm 1.6) \times 10^{-17}$. When this shift is interpreted as a measurement of the change in height of the Al-Mg clock, the result of 37 ± 15 cm agrees well with the known value of 33 cm.

Although ideally $\langle v_\parallel \rangle = 0$, small linear velocities of the Al^+ ions can occur because of effects such as slow electrical charging of insulating material in the trap. From Eq. 1, the clock's frequency (that is, the frequency of the probe laser locked to the moving ion's clock transition) exhibits a fractional frequency shift

$$\frac{\delta f}{f_0} \approx \frac{\langle v_\parallel \rangle}{c} \qquad (3)$$

if the Al^+ ion is moving at an average velocity $\langle v_\parallel \rangle$ in the propagation direction of a probe laser beam. In the comparison measurements between the Al^+ clocks, the Doppler effect was carefully constrained by alternate use of probe laser beams counter-propagating with respect to each other (*11*). Any motion of the ion is detected as a difference in the transition frequencies measured by the two laser beams. In the Al-Mg clock, we observed a fractional frequency difference of $(1.2 \pm 0.7) \times 10^{-17}$ between the two probe directions, which corresponds to the ion moving at a speed of (1.8 ± 1.1) nm/s in the lab frame. However, the clock rate is not significantly affected by a velocity of this magnitude, because it is derived from an average of the two opposite laser-probe directions.

Small relativistic effects reported here have been observed with optical atomic clocks of unprecedented precision and accuracy. With improved accuracy, the sensitivity of optical clocks to small variations in gravitational potential might find applications in geodesy (*19*, *20*), hydrology (*21*), and tests of fundamental physics in space (*22*). The basic components for clock-based geodetic measurements were demonstrated here by comparing two accurate Al^+ optical clocks through 75 m of noise-canceled fiber and measuring height-dependent clock shifts. In clock-based geodesy (*23*, *24*), accurate optical clocks would be linked to form a network of "inland tide gauges" (*25*) that measure the distance from Earth's surface to the geoid: the equipotential surface of Earth's gravity field that matches the global mean sea level. Such a network could operate with high temporal (daily) and geospatial resolution at the clock locations. It would therefore complement geodetic leveling networks, whose update period is typically 10 years or longer, as well as biweekly satellite-generated global geoid maps.

For a network to be useful, clock accuracy must be improved to 10^{-18} or better (*26*–*28*) to allow for height measurements with 1-cm uncertainty. In Al^+ clocks, improved control of the ion motion is needed to reduce the uncertainty of motional time dilation, and issues of reliability must be addressed, so that the clocks can operate unattended for long periods. High-quality links are also needed to connect the optical clocks. Realistic link demonstrations with telecommunications fiber akin to the links used in this work have shown that optical frequencies can be transmitted across fiber lengths of up to 250 km with inaccuracy below 10^{-18} (*29*–*31*), and continent-scale demonstrations are in progress (*30*). However, intercontinental links may require the faithful transmission of optical carrier frequencies to satellites through the atmosphere, and this is an unsolved problem under active investigation (*32*, *33*).

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Supporting Online Material

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Methods
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Alkali-Stabilized Pt-OH_x Species Catalyze Low-Temperature Water-Gas Shift Reactions

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We report that alkali ions (sodium or potassium) added in small amounts activate platinum adsorbed on alumina or silica for the low-temperature water-gas shift (WGS) reaction ($H_2O + CO \rightarrow H_2 + CO_2$) used for producing H_2. The alkali ion–associated surface OH groups are activated by CO at low temperatures (~100°C) in the presence of atomically dispersed platinum. Both experimental evidence and density functional theory calculations suggest that a partially oxidized Pt-alkali-$O_x(OH)_y$ species is the active site for the low-temperature Pt-catalyzed WGS reaction. These findings are useful for the design of highly active and stable WGS catalysts that contain only trace amounts of a precious metal without the need for a reducible oxide support such as ceria.

The water-gas shift (WGS) reaction is a key step in all carbon-based fuel processing aimed at producing and upgrading hydrogen (*1*). For use in low-temperature fuel cells, the WGS catalysts must be nonpyrophoric, active over a wide temperature range, and stable during frequent shutdown-restart operation cycles. To meet these criteria, a reemergence of platinum-group metals has occurred in recent years, and various combinations are being evaluated as low-temperature WGS catalysts (*2*). Nanoscale ceria is the support of choice because of its high concentration of oxygen defects and stabilization of active Pt species (*2*).

The WGS reaction mechanism on Pt-CeO_2 has been debated as either involving formate intermediates (*3*) or a simple redox chemistry (*4*). Recently, Mavrikakis and co-workers showed that a carboxyl-mediated mechanism is responsible for the WGS reaction on Cu (*5*) and other metallic sites, including Pt (*6*). In both the redox and carboxyl mechanisms, the activation of H_2O, leading to the formation of the species responsible for oxidizing CO, is a kinetically important step (*7*). Furthermore, the supply of oxidizing species and the related WGS activity has been shown to be high on metals supported on reducible oxides (*8*), whereas no WGS activity has been reported for Pt supported on irreducible oxides, such as silica.

A recent key finding is that oxidized Pt (and Au) species in ceria are the catalytic sites for the WGS reaction (*2*, *9*); Pt or Au nanoparticles are spectator species. This observation has also been made for Au on Fe_3O_4 catalysts (*10*, *11*). On these oxide surfaces, nanoparticles, clusters, and atoms (ions) of Pt or Au coexist, but the chemistry takes place on the atomically dispersed $Pt^{\delta+}$ or $Au^{\delta+}$ species and involves the neighboring OH groups. Aberration-corrected high angle annular dark field (HAADF) scanning transmission electron micros-

Table 1. Physical properties and reducibility of parent and washed (deionized water–washed samples were heated to 400°C in air 4 hours) xPt-yNa-SiO_2 samples (x and y are atomic percentages.). Bulk composition was measured by inductively coupled plasma optical emission spectrometry; surface concentration, by XPS; surface [O], from CO-TPR (5% CO–He, from room temperature to 400°C, at 10°C/min); and surface [OH] from CO-TPR using only the CO_2 peak associated with the production of H_2. The XPS-measured value of surface Pt was used in calculating the [OH]/Pt ratio. Dash entries indicate nondetectable amounts, and NA, not applicable.

Sample	Bulk composition (atomic %)		Surface concentration (atomic %)		Surface [O] ($\mu mol/g_{cat}$)	Surface Na/Pt	Surface Na/O	Surface [OH]/Pt
	Pt	Na	Pt	Na				
3Na-SiO_2	0	3.0	0	4.0	500	NA	1.5:1	NA
3Na-SiO_2 washed	0	–	0	–	–	NA	–	NA
1Pt-1Na-SiO_2	1	1.0	0.3	0.9	210	3:1	0.7:1	2:1
1Pt-1Na-SiO_2 washed	1	0.7	0.4	0.5	185	1.2:1	1:1.5	2:1
1Pt-3Na-SiO_2	1	3.0	0.3	3.9	450	13:1	1.4:1	3:1
1Pt-3Na-SiO_2 washed	1	1.6	0.4	1.2	245	3:1	1:1	3:1

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copy (STEM) has been used to identify atoms, clusters, and nanoparticles of Au on FeO_x (*11*, *12*) as well as Pt atoms and clusters on γ-Al_2O_3 (*13*), in both cases after a prolonged high-temperature annealing. On the basis of such models, we set out to show that, in order for a Pt atom to become active for the WGS reaction, it must be stabilized in an oxidized form and be surrounded by reactive OH groups. To avoid the usual implication of the support in the mechanism, we examined inert supports and used the alkali ions to stabilize OH species in the vicinity of Pt atoms.

An alkali promotion effect on Pt-based catalysts for the low-temperature WGS reaction has been reported for Pt on reducible or partially reducible oxides, such as titania (*14*, *15*), zirconia (*16*), and ceria (*17*), but the interpretation is complicated by the nonzero activity of the alkali-free state. The promotion has been explained previously as an alkali-induced C-H bond weakening in formates, the latter assumed to be the key reaction intermediates (*17*). However, formates have been shown to be spectator species for the WGS and reverse-WGS reactions on Pt catalysts (*18*, *19*). The same conclusion for Pt catalysts has been reached by a combination of experiments and density functional theory (DFT)–based microkinetic modeling (*6*).

In 1981, Amenomiya and co-workers (*20*) reported that addition of potassium rendered γ-alumina a high-temperature WGS catalyst. Reducible surface oxygen and a large excess of surface hydroxyl groups were found on K-promoted alumina, and the catalytic activity increased with the concentration of K^+ ions. We show that adding a metal such as Pt to the alkali-promoted alumina or silica surface has the same effect as adding a metal to ceria; that is, the surface oxygen associated with the metal and alkali ions becomes reducible at low temperatures, and the composite is an excellent low-temperature WGS catalyst.

The properties of alkali-promoted catalyst samples prepared by different methods, as detailed in (*21*), were insensitive to the preparation method and to the sequence of Pt and alkali ion addition when a sequential incipient wetness impregnation was used. The presence of alkali suppresses the metal particle growth during the heat treatment step (*22*). Furthermore, we found that sub-nanometer Pt clusters and atoms present on the Na-modified silica surfaces were stable after several heat treatments in air at 400°C. This is shown in the HAADF/STEM images and corresponding particle size distributions of Pt-SiO_2 samples (Fig. 1 and fig. S1A). After calcination, the sample shown in Fig. 1B had been washed to remove excess Na and annealed again at 400°C for 4 hours in air, that is, for twice as long as the Na-free sample (Fig. 1A). The sub-nanometer Pt clusters and atoms were abundant (65%) in the Na-promoted sample, Fig. 1B, but scarce (5%) in the Na-free sample, Fig. 1A. Metallic Pt was found by x-ray diffraction (XRD) in the latter but not in the former (fig. S2). No bulk Na_2SiO_3 was detected in either sample by XRD.

X-ray photoelectron spectroscopy (XPS) of the Na-promoted and Na-free samples showed that the former had a dramatically smaller fraction of metallic Pt (fig. S3A). The same observations were made for the K-promoted Pt samples. Not all of the Pt on the sample surfaces was XPS-visible, as shown in Table 1, which we attributed to the presence of some large Pt particles as well

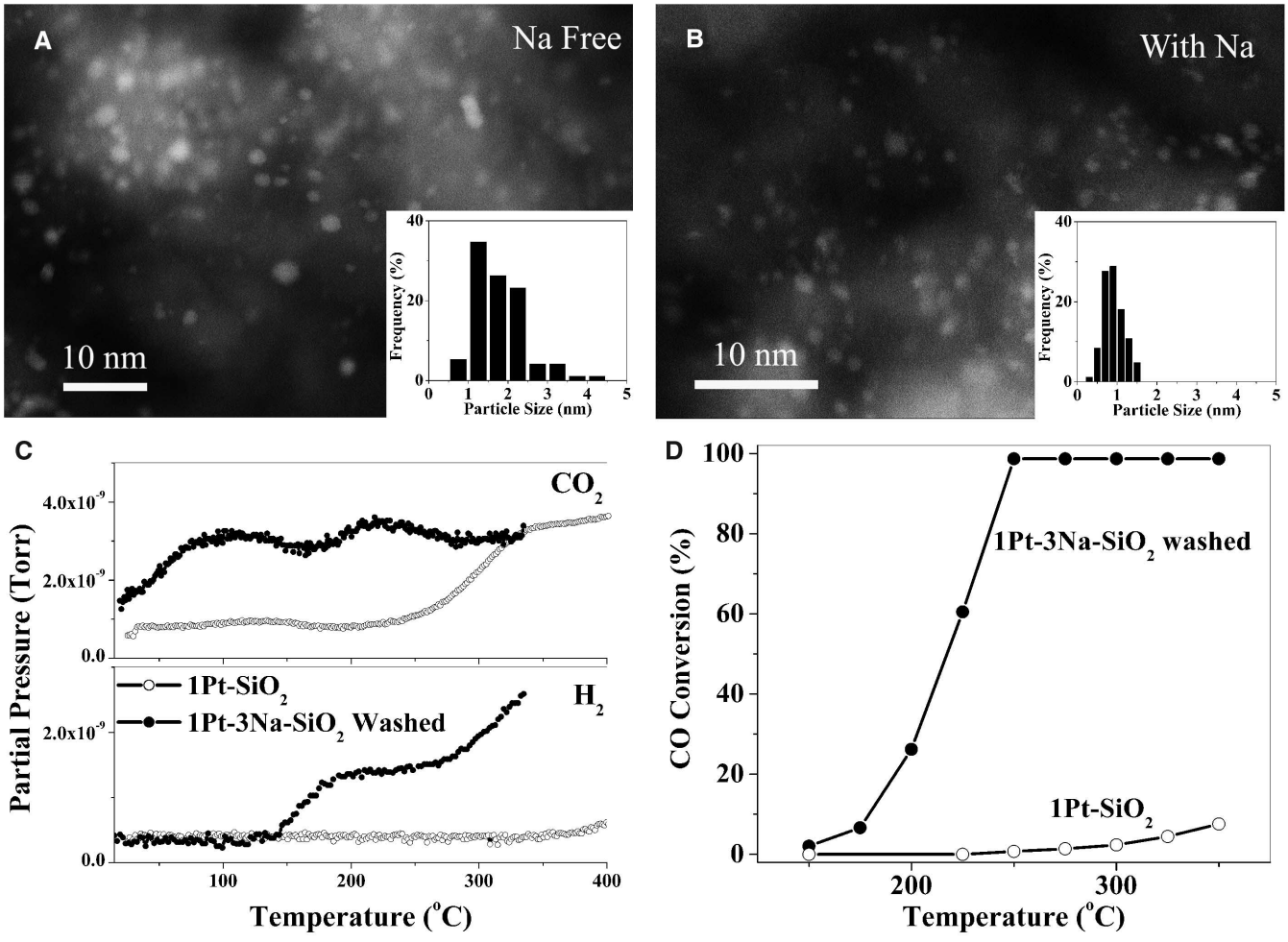


Fig. 1. HAADF/STEM images and particle size distributions (insets, 100 particles counted) of (**A**) 1Pt-SiO_2 and (**B**) 1Pt-3Na-SiO_2 washed samples. (**C**) CO-TPR of samples shown in (A) and (B); 5% CO–He, 20 cm^3/min, with ramping rate at 5°C/min. (**D**) WGS profiles (steady-state data) measured over the samples shown in (A) and (B) in 2% CO–10% H_2O–He gas mixture, contact time of 0.09 g s/cm^3.

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as to the possible coverage of Pt by multiple alkali layers. X-ray absorption near-edge structure (XANES) Pt-L_{III}–edge spectra (fig. S4A) corroborated the highly oxidized state of Pt in the alkali-promoted samples. Hence, the alkali ions stabilize atomically dispersed, oxidized platinum species on the silica surface. Such species have been reported to catalyze the WGS reaction on Pt-CeO_2 (*2*, *9*).

CO–temperature programmed reduction (CO-TPR) was used to identify reducible surface oxygen species and probe the onset of the WGS reaction by the activation of the surface hydroxyls to produce H_2 (*2*). Reducible oxygen is present that reacts to form CO_2 above 200°C (Fig. 1C), and only a negligible number of OH groups are activated above 320°C on the Na-free 1Pt-SiO_2. On the Na-promoted sample, reducible surface oxygen and reactive OH groups are both available at low temperatures (Fig. 1C). These findings were corroborated in steady-state WGS experiments with the same two catalyst samples (Fig. 1D). Thus, although Na-free Pt-SiO_2 contains a small fraction of sub-nanometer Pt clusters and atoms in its as-calcined form (Fig. 1A), it lacks the OH groups present in the Na-promoted Pt-SiO_2 sample, which renders it inactive for the low-temperature WGS reaction.

As detailed in the supporting online material (SOM) and previously reported (*20*), alkali ions promote the WGS reaction activity of bare γ-Al_2O_3 only at temperatures >350°C, whereas no activity was observed for either the Na-free or Na-containing SiO_2. A dramatic promotion of the activity of Pt-Al_2O_3 and Pt-SiO_2 is observed by addition of Na or K ions (Fig. 1D and fig. S5). Interestingly, these materials are as active as CeO_2-supported Pt catalysts (*2*, *9*) for low-temperature WGS. No activation (hysteresis) or deactivation of the catalysts was observed during ramping up and down the temperature. Temperature-programmed oxidation of the used samples found no evidence of carbon deposition. A sample of 1Pt-3Na-SiO_2 used at 275°C for 1 hour in 1% CO–3% H_2O–He gas mixture still contained oxidized Pt as determined by in situ XANES (fig. S4B).

Further experiments were conducted to understand how alkali ions are associated with Pt on the oxide supports. A deionized-water washing procedure was used on selected Na-promoted Pt-SiO_2 samples. The interaction of NaO_x with SiO_2 or Al_2O_3 must be weak because when the Na-containing, Pt-free supports (after the 400°C-air calcination) were washed all of the Na was removed. When the corresponding Pt-containing samples, heat-treated the same way, were washed, part of the Na ions remained, which showed that only the PtO_x-associated alkali fraction is insoluble. Any residual alkali is then attributed to strong Pt-$(ONa)_x$ interactions. Table 1 shows the residual amounts of Na for the same Pt content on SiO_2 from two parent samples containing 3/1 and 1/1 atomic ratios of Na/Pt. No Pt was lost during washing. The 1/1 Na/Pt ratio did not provide enough Na ions to interact with the Pt, which explains why the WGS reaction activity of the 1/1 sample is inferior to that with Na/Pt = 3/1 (fig. S5). XPS analysis found no change in the oxidation state of Pt before and after washing (fig. S3B).

By comparing the CO-TPR of the parent (fig. S7) and washed 1Pt-3Na-SiO_2 (Fig. 1C), we found that the washing step removed part of the high-temperature surface oxygen bound to the extra Na in the parent sample. The onset of H_2 production and the total amount of H_2 produced were the same for both the parent and the washed catalysts, which also shows that the active OH sites are associated with the Pt-O_x-Na species. Notably, parent and washed samples showed identical WGS reactivity in a realistic fuel gas stream (fig. S6). Thus, a certain number of Na atoms interact directly with Pt and cannot be removed with a simple water wash. Any Na ions beyond this number play no role in the Pt active sites; they are weakly bound and washable.

The amount of surface oxygen remaining after washing corresponds to active oxygen and leads to a Na/O atomic ratio = 1/1 (surface oxygen amount was measured by CO-TPR, Table 1). Because the surface Pt amount measured with XPS (Table 1) includes contribution from Pt nanoparticles along with the atomically dispersed Pt, we cannot use the surface Na/Pt ratio measured by XPS (Table 1) as the actual Na/Pt atomic ratio. We can only use it as a lower bound; on this basis, the active sites comprise at least three Na atoms bound to each Pt atom. By integrating the amount of oxygen reduced in the range of 100° to 300°C, we calculate a value of OH/Pt equal to between two and three (Table 1).

To examine in more detail the nature of the active site for the low-temperature WGS reaction, we performed DFT calculations by using the VASP code (*23*) based on the generalized gradient approximation (*24*) and the projector augmented-wave method (*25*, *26*). [The computational methods are detailed in (*21*).] We aimed to limit the possibilities for the composition of the active site by constraining the possible phase space with constraints provided by our experimental findings. We first looked at the relative stability of added alkali atoms to a Pt atom. Following a similar approach as has been used to identify stable vanadium-cesium VCs_x superatoms (*27*), we added more K atoms incrementally to the central Pt atom of the site and calculated the differential binding energy of the last K atom to be added to the cluster site. After the initial addition of two K atoms to the core Pt atom of the cluster site, the differential binding of further K did not vary much (Fig. 2A), but there was a local binding energy minimum at PtK_6. Thus, PtK_6 was used as the basis for a model of the WGS active site on alkali-promoted Pt.

A charge analysis performed for all possible site structures (Fig. 2B) verified that K atoms donate electrons to Pt. Thus, to create the Pt^{2+} and Pt^{4+} seen with XPS, electron-withdrawing

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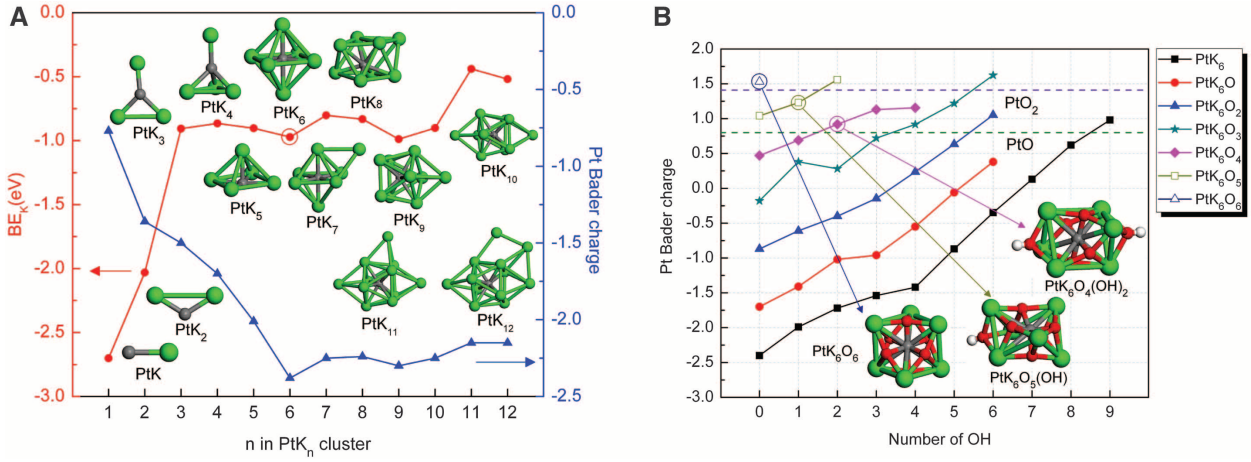


Fig. 2. (**A**) Relative stability (red line), geometric structure, and Bader charge of Pt (blue line) in PtK_n clusters. Pt and K atoms are shown in gray and green spheres, respectively. (**B**) Bader charge of Pt in $PtK_6O_x(OH)_y$ cluster sites. Bader charge of Pt on bulk PtO and PtO_2 (α phase) are given by the dashed horizontal lines for reference. O and H are shown in red and white spheres, respectively. Circled are active sites that are more thoroughly explored (see SOM).

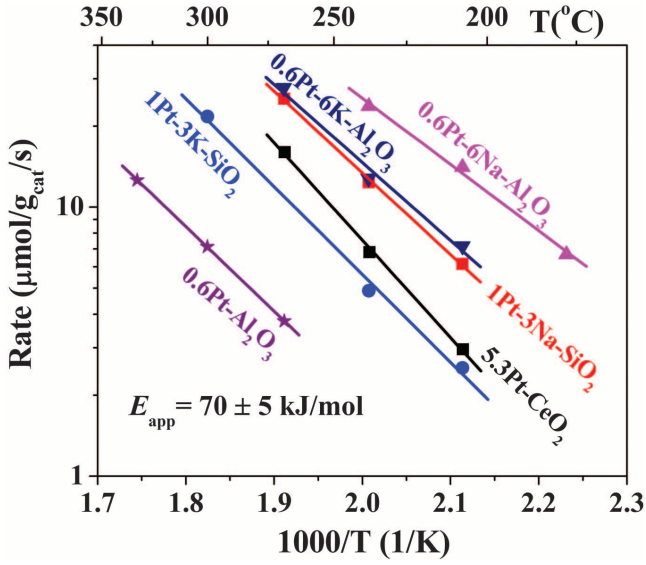


Fig. 3. WGS reaction rates over Pt-based catalysts measured in 11% CO–26% H_2O–26% H_2–7% CO_2–He. Cat, catalyst.

groups, such as O and/or OH groups, were added to the PtK_6 site. Such oxide species could occur on highly dispersed Pt (*13*). As references, the calculated Bader charges on Pt in bulk PtO and PtO_2 (α phase), both references used in the XPS analysis, are 0.80 and 1.41, respectively. Thus, we systematically studied several possible cluster sites of the general composition PtK_6O_x and $PtK_6O_x(OH)_y$ to identify sites with Pt-charge characteristics similar to those of Pt in bulk PtO and PtO_2 and established a narrow set of candidate active-site structures (Fig. 2B).

Calculations were performed to identify the most promising active site model, which matches the K/O ratio, Bader charge on Pt, and thermochemical properties relevant to key steps in the WGS reaction. These thermochemical predictions for the various $PtK_6O_x(OH)_y$ sites were compared with those for Cu(111) and Cu(211) extended surfaces because Cu is one of the best low-temperature shift catalysts and these are the properties that are appropriate to mimic. Further, by comparing with the respective properties of a Pt(111) extended surface, the thermochemical changes induced to the Pt by the combination of its atomic dispersion and the presence of alkali and O/OH species in the immediate vicinity of the site can be demonstrated. On the basis of this analysis (table S2), $PtK_6O_4(OH)_2$ was identified as the most promising candidate for the active low-temperature WGS reaction site. Compared with Cu(111), $PtK_6O_4(OH)_2$ binds additional OH slightly weaker and the thermochemistry of water activation is only mildly more exothermic, whereas it binds CO moderately. Additionally, the charge on the Pt center of the site is close to the charge of Pt in PtO, corresponding to the Pt^{2+} oxidation state. Although other active sites may fulfill the same role as $PtK_6O_4(OH)_2$, they must capture the same essential elements that this candidate does, including thermochemistry of binding of various intermediates and Pt oxidation state. It is possible that a mixture of partially oxidized Pt-alkali sites is responsible for the remarkable chemistry observed here.

In kinetic tests, we found that all the alkali-promoted samples on the alumina or silica supports have similar apparent activation energy, 70 ± 5 kJ/mol (SD) (Fig. 3). The difference in the reaction rates is caused by the different number of Pt active sites (*28*). Interestingly, the same apparent activation energy was measured on an alkali-free $Pt-CeO_2$ sample (*2*). A thorough check of the reported values shows that the apparent activation energy of the WGS reaction on Pt-based WGS catalysts, for example, $Pt-CeO_2$ (*2*, *9*, *29*), $Pt-TiO_2$ (*30*), $Pt-Al_2O_3$ (*6*, *29*), $Pt-ZrO_2$ (*31*), is similar, 70 ± 5 kJ/mol, irrespective of the support used. Hence, the alkali promotion can be understood as a means of stabilizing and providing the oxygen-containing (OH) species to the Pt atoms. These OH groups are not present on the alkali-free alumina or silica surfaces, unlike on the reducible ceria.

On the basis of these findings, we propose that the key steps of the WGS reaction catalysis (CO adsorption and H_2O activation) occur on oxidized Pt sites. The presence of the alkali or Ce ions is important to stabilize the active Pt sites, but the host oxide does not participate in the reaction. A common active site appears to be operating on all Pt-based catalysts for the WGS reaction irrespective of the nature of the support.

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Supporting Online Material

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Materials and Methods
SOM Text
Figs. S1 to S8
Tables S1 and S2
References

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Graphene Double-Layer Capacitor with ac Line-Filtering Performance

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Electric double-layer capacitors (DLCs) can have high storage capacity, but their porous electrodes cause them to perform like resistors in filter circuits that remove ripple from rectified direct current. We have demonstrated efficient filtering of 120-hertz current with DLCs with electrodes made from vertically oriented graphene nanosheets grown directly on metal current collectors. This design minimized electronic and ionic resistances and produced capacitors with RC time constants of less than 200 microseconds, in contrast with ~1 second for typical DLCs. Graphene nanosheets have a preponderance of exposed edge planes that greatly increases charge storage as compared with that of designs that rely on basal plane surfaces. Capacitors constructed with these electrodes could be smaller than the low-voltage aluminum electrolyte capacitors that are typically used in electronic devices.

Electric double-layer capacitors (DLCs), also called supercapacitors or ultracapacitors, store charge in the double layer formed at an electrolyte-electrode interface when voltage is applied. The electrodes are generally composed of high-surface-area conductive material, usually activated carbon (*1*). DLCs typically store more than an order of magnitude more energy per unit volume than conventional capacitors (*2*) but, like batteries, are low-voltage devices, so they often must be connected in series to meet system voltage requirements. Electric double-layer charge storage was first observed more than 100 years ago, but DLC products did not reach the market until 1978 (*3*). The introduction of volatile computer memory created the need for a power source that could be charged repeatedly and then reliably deliver low levels of dc power over a long time. Kilofarad-sized capacitors became available starting in the mid-1990s to "load-level" the power profile of battery-powered electric vehicles that were then under development (*4*) and, more recently, to capture and store energy derived from kinetic energy harvesting—for instance, the braking energy of a hybrid vehicle (*5*).

The delayed introduction of DLCs was caused by a limited market for capacitors that could only store charge but performed poorly at their other main task: filtering voltage ripple (*6*). The typical resistor-capacitor (RC) time constant for a DLC is ~1 s—far too long to be useful for the common application of 120-Hz filtering (8.3 ms period), which entails smoothing the leftover ac ripple on dc voltage busses found in most line-powered electronics. Sixty-Hz ac power is full-wave rectified and then filtered to create pure dc voltage. Filtering today is performed primarily by means of aluminum electrolytic capacitors, which usually are among the largest components found in any electronic circuit: Smaller-sized filtering capacitors may allow system size reductions, which is particularly valuable in some portable electronics applications.

Present DLCs have an impedance phase-angle at 120 Hz that is near 0°, which is far from the –90° value needed for filtering. This drawback is a direct result of using porous electrodes, which store charge in a distributed fashion. When driven by 120-Hz ac, they respond like a transmission line (*7*); that is, they behave like a resistor rather than a capacitor. Thus, high-surface-area materials with less inherent porosity have been examined. DLCs fabricated from felt-like electrodes comprising entangled, multiwall carbon nanotubes (CNTs) did establish a frequency response record in 1997: 6 Hz for an impedance phase angle of –45° (*8*). (Resistance and reactance have equal magnitudes at a phase angle of –45°, making this frequency convenient for comparison purposes.) These DLCs had thin electrodes and used a high-conductivity aqueous electrolyte but were still incapable of filtering at 120 Hz for two reasons. First, the felt-like structure created porosity with a pore length equal to the electrode thickness, thus creating distributed charge storage with associated ionic resistance. Second, the structure relied on manifold electrical contacts among the individual CNTs and against the current collector, contributing to the electronic resistance. Although these capacitors were unable to filter, they showed that DLC frequency response could be improved by using electrode materials with external rather than internal surface area.

Further improvements in frequency response were reported from electrodes of multiwall CNTs that were deposited onto a metal current collector, cleared of oxygen surface groups, and bonded to the collector with a hydrogen furnace treatment (*9*–*11*). These capacitors reached a –45° phase angle at 636 Hz, which is more than 100 times the 1997 record. The phase angle at 120 Hz was –65°, and thus they could not efficiently filter at this frequency. Multi-wall, vertically aligned CNTs grown directly on a metal current collector were also investigated as DLC electrodes. One such study showed impedance data with a –45° phase angle at 443 Hz (*12*). Yet another study bonded vertically aligned CNT electrodes to an aluminum current collector, but this also was unable to efficiently filter 120-Hz ripple (*13*).

Agglomerated, chemically modified graphene material was evaluated as a DLC electrode and

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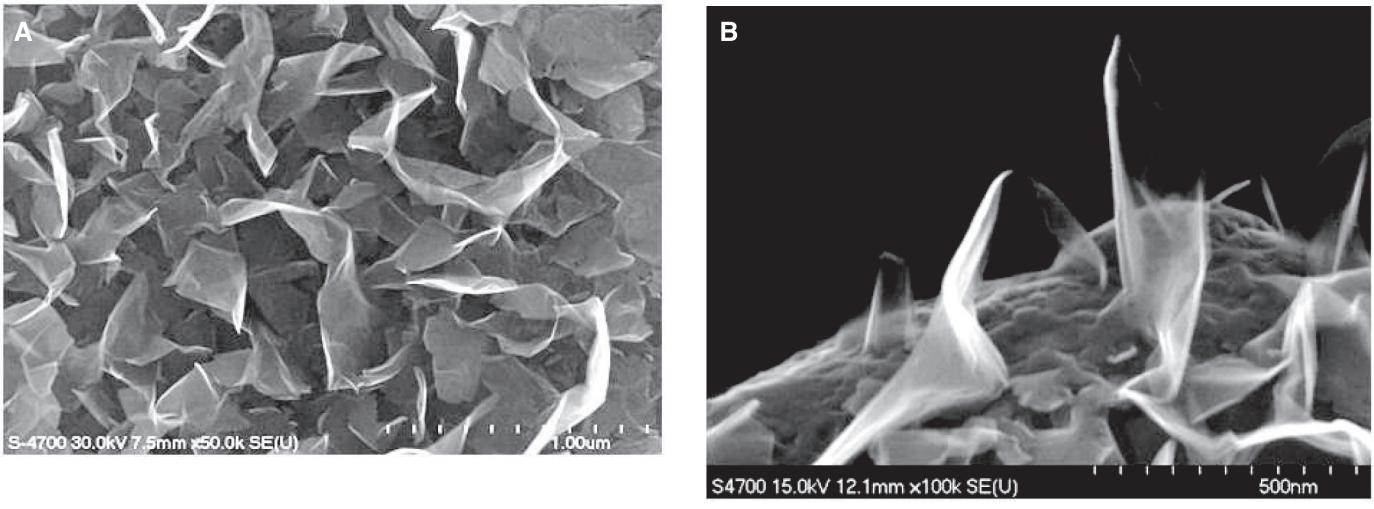


Fig. 1. (**A**) Plan SEM micrograph of coated Ni electrode. (**B**) SEM micrograph of a coated fiber, showing plan and shallow-angle views.

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showed a considerable degree of unwanted porous-electrode behavior (*14*). Apparently, direct access to the charge-storage surfaces was severely restricted by the structure, adding substantial ionic resistance. Furthermore, the bonded electrode relied on multiple electrical contacts among the graphene agglomerates, similar to the contacts present in all particulate electrodes, thus contributing additional electronic resistance. Capacitors constructed with electrodes of agglomerated graphene electrode as reported were not capable of 120-Hz filtering.

Electrodes with vertically oriented graphene nanosheets coating carbon fibers have also been examined (*15*). Capacitor designs were developed for a 14,900 F device, but no two-terminal electrical responses were reported. Such vertically oriented graphene nanosheets are believed to offer a near-ideal structure for DLC electrodes capable of high-frequency operation. First, they have a preponderance of edge planes that provide capacitance of 50 to 70 μF/cm^2 in comparison with that of basal planes, which provide capacitance of only ~3 μF/cm^2 (*16*). Second, charge-storage edge planes are exposed and directly accessible, minimizing the distributed nature of the charge storage. Third, porosity effects are minimal because of the open structure, reducing ionic resistances. And fourth, graphene nanosheets themselves have extremely high conductivity and can be grown from a conductive surface, minimizing electronic resistances. These factors, we expect, should provide high levels of charge storage that is accessible through minimum series resistance and thus allow creation of a DLC capable of high-frequency operation.

We synthesized vertically oriented graphene nanosheets directly on heated nickel (Ni) substrates using radio frequency (RF) plasma–enhanced chemical vapor deposition (*17*, *18*). After pump-down, the substrates were plasma-etched for 10 min in 40% argon (Ar) + 60% H_2 (total pressure of 50 mT). After the Ar was shut off, the Ni substrate was heated to 1000°C in H_2, methane (CH_4) was added, and a 1000-W plasma was generated by using 40% CH_4 and 60% H_2 at a total pressure of 85 mT. Initially, graphitic islands formed on the Ni substrate (Volmer-Weber planar growth), with two-dimensional growth continuing until impingement with other islands. This basal layer was approximately 10 to 15 nm thick. After 20 min of growth, the resulting coating was composed of vertically oriented graphitic nanosheets approximately 600 nm high with a cross section <1 nm thick, but often terminating with an edge of a graphene sheet (one atomic layer).

A plan view of vertically oriented graphene nanosheet electrode (Fig. 1A) shows the irregular morphology of the surface (exposed edge planes and the random expansive open areas) created by defects that arise from stress and hydrogen incorporation. The structures grow vertically from the metal substrate and are electronically connected to it. The measured surface area was ~1100 m^2/g. Similar graphene growth is shown in Fig. 1B, but on a circular fiber so as to display both plan and shallow-angle views of the surface. Raman intensity measurements of the coating had a D/G band intensity ratio (I_D/I_G) of 0.67 because of defects.

Capacitors were fabricated to measure the electrical performance of the vertically oriented graphene nanosheet electrodes. Two identical 2.5-cm-diameter, 75-μm-thick Ni disks with graphene nanosheet coatings grown over their 1.6-cm-diameter central region (2.0 cm^2 area) were separated by a 25-μm-thick microporous separator. The coatings and the separator were wetted with the aqueous electrolyte [25% potassium hydroxide (KOH)] before sealing the perimeter of the disks with a thermoplastic by use of an impulse-heat-seal apparatus. These packaged prototypes, 2.5 cm diameter by ~175 μm thick, had a mass of ~0.8 g. The active material-coating thickness on each electrode was approximately 0.6 μm, which is negligible compared with device dimensions and mass. Electrical connection was made to the back surface of each Ni disk.

Impedance phase angle data of a prototype capacitor fabricated with the vertically oriented graphene nanosheet electrodes is shown in Fig. 2. Data from a commercial DLC and from an aluminum electrolytic capacitor are included for comparison. All sets of data show capacitive behavior (near –90° phase angle) at low frequency and inductive behavior at high frequency. However, the transition between these two limits occurs over a single decade in frequency for the graphene nanosheet capacitor, whereas it occurs over approximately seven decades in frequency for the activated carbon capacitor. This disparity is due almost entirely to electrode porosity differences. The impedance phase angle of the graphene nanosheet capacitor reached –45° at ~15,000 Hz in comparison with 0.15 Hz for the activated carbon capacitor and ~30,000 Hz for the electrolytic capacitor. At 120 Hz, the impedance phase angle of the graphene nanosheet capacitor was approximately –82° as compared with ~0° for the activated carbon capacitor and approximately –83° for the aluminum electrolytic capacitor. The phase angle for a blank (bare Ni electrode prototype) was –85°.

A complex plane plot of the impedance data obtained from the vertically oriented graphene nanosheet capacitor is shown in Fig. 3, with an expanded view in the inset. There is no evidence of porous electrode behavior, which would manifest itself by a line that intersects the real axis at a near-45° angle. There are also no features associated with a series-passive layer (high-frequency semicircle), which would add series resistance. Data fit a near-vertical line as produced by a series-RC circuit. Raistrick modeled the impedance of rough, saw-tooth-surface electrodes having various height-to-separation ratios (*19*). Fig. 3 follows such rough-surface behavior with an aspect ratio of <2, which is consistent with the observed scanning electron microscopy (SEM) micrograph structure.

We used a series-RC circuit model in which resistance is the real part of the impedance and capacitance is calculated as $C = -1/(2\pi f Z'')$, where f is frequency in Hz and Z'' is the imag-

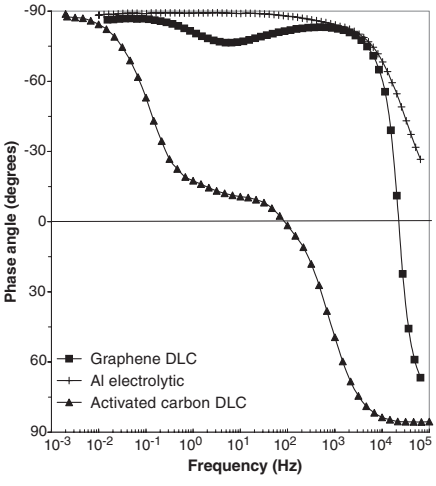


Fig. 2. Impedance phase angle versus frequency for the graphene nanosheet DLC. Measurements from a commercial DLC having an activated carbon electrode and an aluminum electrolytic capacitor are shown for comparison.

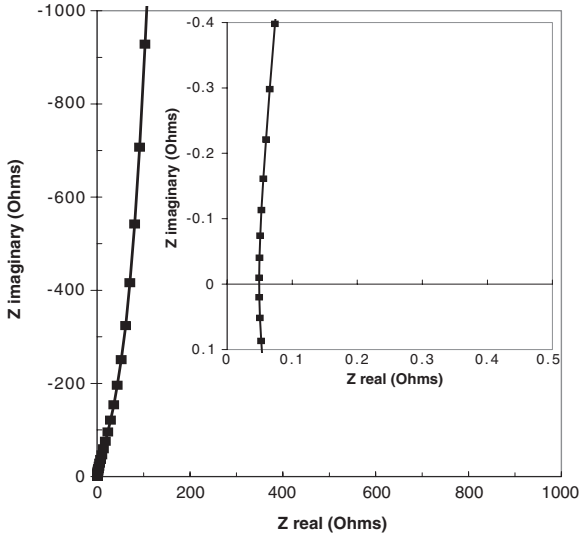


Fig. 3. Complex plane plot of the impedance of the graphene nanosheet capacitor showing vertical intersection with the real axis and not the usual porous electrode behavior shown by DLCs.

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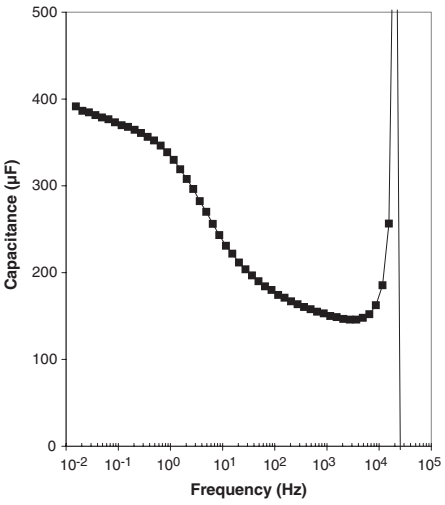


Fig. 4. Capacitance versus frequency of the graphene nanosheet DLC, assuming a series-RC circuit model. Capacitive behavior is shown up to ~10^4 Hz.

inary part of the impedance. Capacitance versus frequency from this equation is plotted in Fig. 4 for the graphene nanosheet capacitor shown in the previous figures. At 120 Hz, the derived capacitance value is 175 μF, and the measured resistance is 1.1 ohms, yielding an RC time constant of less than 200 μs. Divergent behavior near 20 kHz is an artifact of the model (where Z″ passes through zero) and should be ignored. As shown, the capacitance value increases from ~175 μF in the 10- to 10^4-Hz band up to ~375 μF at frequencies below ~1 Hz. The increase occurs in the region of the phase angle dip shown in Fig. 3. Capacitance saturation (horizontal behavior) was not observed even at 0.01 Hz, although this device exhibited incredibly fast response, suggesting the involvement of a second, much-lower-rate charge-storage process. Three-electrode measurements revealed that the positive electrode had greater capacitance increase at low frequency than did the negative electrode. Capacitors of the same design fabricated by using bare Ni electrodes had capacitance values of less than 25 μF at 120 Hz. We attribute the marked capacitance increase at low frequency to pseudocapacitance derived from ion intercalation into the exposed edge planes of the graphene structure. Pseudocapacitance by this mechanism has been reported for exfoliated carbon fibers with sulfuric acid electrolyte (*20*).

A similar vertically oriented graphene nanosheet capacitor fabricated with an organic electrolyte (1 M tetraethylammonium-tetrafluoroborate salt in propylene carbonate) showed more definite but still incomplete saturation of capacitance at low frequency (fig. S1). The organic electrolyte capacitance was ~50% higher than the value from aqueous electrolyte prototypes, which is opposite to typical activated carbon behavior. This boost in capacitance using the organic electrolyte may result from more complete wetting of the graphene nanosheet surface with this electrolyte.

Capacitance at 120 Hz was ~175 μF for the graphene nanosheet capacitor (with KOH electrolyte), which corresponds to a capacitance density of the 0.6-μm-thick active layer of ~3 F/cm^3. The graphene active layer stored ~1.5 FV/cm^3 with the aqueous electrolyte (0.5 V) and ~5.5 FV/cm^3 with the organic electrolyte (1.25 V). Aluminum electrolytic capacitor foil is highly etched before being anodized so as to create charge storage throughout its volume. Low-voltage aluminum anode foil (KDK, Tokyo, Japan) has CV/volume values up to ~0.14 FV/cm^3. Thus, a graphene DLC in principal could have substantially smaller volume than a comparably rated low-voltage aluminum electrolytic capacitor.

DLC designs would differ from the spiral-wound construction commonly used with aluminum electrolytic capacitors because of operating voltage differences. A bipolar design (cell stacking), as presently used by several DLC manufacturers, offers volumetric efficiency and may be optimal. With stacked cells, DLC capacitance density scales like the inverse of the square of the number of series-connected cells: ~$1/V^2$, where V is the device operating voltage. Capacitance density of an electrolytic capacitor scales like 1/V because the CV product of anodic dielectric is approximately constant. Thus, DLC capacitance density advantages will disappear at a particular voltage. Using prototype performance measurements with conventional construction materials in a practical design (fig. S2), a single-cell graphene DLC (~2.5 V operation) should offer a sixfold or greater volume advantage over an aluminum electrolytic capacitor of the same rating. A two-cell graphene DLC (~5 V operation) should offer a twofold or greater volume advantage over an equivalently rated aluminum electrolytic capacitor. No volumetric advantages are expected at higher voltage. Aluminum electrolytic capacitors rated at 2 V or higher are widely used today for bypass and filtering in portable electronics equipment.

Graphene nanosheet electrodes could be manufactured by using standard semiconductor process equipment. The fabrication of the electrode and the choice of electrolyte have not been optimized, and increases in capacitance density through further optimization appear likely. Cell operating voltage may be increased with ionic liquid electrolytes or by use of an asymmetric design, with either approach expanding the voltage region in which the graphene DLC capacitance density exceeds that of present aluminum electrolytic capacitor technology.

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Figs. S1 and S2

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Slip Systems in $MgSiO_3$ Post-Perovskite: Implications for *D″* Anisotropy

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Understanding deformation of mineral phases in the lowermost mantle is important for interpreting seismic anisotropy in Earth's interior. Recently, there has been considerable controversy regarding deformation-induced slip in $MgSiO_3$ post-perovskite. Here, we observe that (001) lattice planes are oriented at high angles to the compression direction immediately after transformation and before deformation. Upon compression from 148 gigapascals (GPa) to 185 GPa, this preferred orientation more than doubles in strength, implying slip on (001) lattice planes. This contrasts with a previous experiment that recorded preferred orientation likely generated during the phase transformation rather than deformation. If we use our results to model deformation and anisotropy development in the *D″* region of the lower mantle, shear-wave splitting (characterized by fast horizontally polarized shear waves) is consistent with seismic observations.

The *D″* region lies just above the core mantle boundary (CMB). In contrast to the bulk of the lower mantle, this ~200-km-thick layer possesses apparent seismic complexity, including seismic discontinuity, large topographic variations, lateral heterogeneity, variable anisotropy, and ultra-

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low velocity zones (*1*–*4*). The *D*″ region is of great interest from a geodynamic perspective because it is a boundary layer between two regions with extreme viscosity contrasts (i.e., the solid mantle and liquid outer core). As such, it should play an active role in controlling mantle convection, thermal structure, and evolution of Earth (*5*). Numerical modeling (*6*) and laboratory experiments (*7*) indicate that deformation is enhanced near boundary layers, and large strain deformation is expected to occur in the *D*″ region, particularly in slabs subducted to the CMB. Thus, it is likely that seismic anisotropies observed in this region are produced by deformation-induced texturing (preferred orientation) of the constituent minerals (*2*, *6*, *7*).

The discovery of a solid-solid phase transition in $MgSiO_3$ from a perovskite (Pv) to a post-perovskite (pPv) structure at conditions closely corresponding to those of the CMB (127 GPa and 2500 K) provided a new perspective for interpreting the *D*″ layer (*8*–*10*). However, several issues remain unresolved, including how texture develops in pPv and how the deformation state in the *D*″ is expressed as seismic anisotropy. Texture may be important in explaining the sharpness of the *D*″ discontinuity (*11*); seismological observations suggest the thickness of the discontinuity to be less than 30 km (*12*), but experimental data predicts that the thicknesses should be on the order of 90 km for an isotropic aggregate (*13*–*15*).

When stress is applied to a polycrystal, individual crystals deform preferentially along slip planes. This results in crystal rotations that in turn lead to preferred orientation of the polycrystal (texture). Because individual crystals are anisotropic, texturing can result in bulk anisotropy. Previous deformation experiments on $MgSiO_3$ pPv and the $MgGeO_3$ pPv analog produced textures consistent with slip on {110} and/or (100) planes (*16*, *17*); however, these slip systems are expected to generate anisotropy largely incompatible with seismic observations. The older experiments showed that texture developed during conversion to the pPv phase and that further compression did not result in textural changes (*16*, *17*). Recently, Okada *et al.* (*18*) performed new experiments on $MgGeO_3$ pPv. After transformation from the Pv to pPv phase, (001) planes were oriented at high angles to compression. When $MgGeO_3$ enstatite was used as a starting material, axial diffraction patterns consistent with the textures of Merkel *et al.* (*16*, *17*) were observed. After further compression, it appeared that (001) planes became aligned normal to compression, implying that the former texture is related to the phase transformation (*18*).

In the $CaIrO_3$ pPv analog, however, the dominant slip plane is (010) over a range of pressures, temperatures, and strain rates (*19*, *20*–*23*). This differing slip system may arise due to bonding differences in $CaIrO_3$ pPv from other pPv structured compounds (*24*). First-principles computations also find $CaIrO_3$ pPv to have elastic properties and an electronic structure that are different from $MgSiO_3$ pPv (*25*), and modeling of dislocation cores predicts that $CaIrO_3$ is much more plastically anisotropic than $MgSiO_3$ pPv (*26*, *27*).

We performed axial compression experiments on $MgSiO_3$ pPv between 148 GPa and 185 GPa in the diamond anvil cell (DAC). The evolution of texture and lattice strains were recorded in situ, using monochromatic synchrotron x-ray diffraction in radial geometry. The starting material of vitreous $MgSiO_3$ was mixed with ~10 weight percent Pt powder to serve as a laser absorber and a pressure standard, and was compressed to high pressure (*28*). Conversion directly to the pPv phase was obtained by laser heating at ~3500 K for ~10 min (*28*). After conversion to pPv, pressure in the sample was 148 GPa (table S2). Pressure was then increased in four steps to 185 GPa over the course of 7 hours (table S2). At each step, in situ radial x-ray diffraction images were collected to document the evolution of pressure, differential stress, and texture.

Radial diffraction images show variations in peak position with respect to the compression direction, which indicate elastic stresses imposed by the DAC, as well as systematic intensity variations, which denote texture. These variations are best visualized if the image is unrolled to display azimuth versus diffraction angle (Fig. 1). To quantitatively extract texture information and calculate differential stress, we use the Rietveld method as implemented in the software package MAUD (*28*, *29*). After conversion, at 148 GPa, differential stress was 5.3 ± 0.1 GPa. At the highest pressure attained, 185 GPa, the differential stress was 10.9 ± 0.5 GPa (table S2). Inverse pole figures (IPF) of the compression direction show the probability of finding the pole (normal) to a lattice plane in the compression direction (Fig. 2). Just after conversion to pPv, the sample exhibits a texture characterized by (001) lattice planes at high angles to compression, with an IPF maximum of 4.09 multiples of a random distribution (m.r.d.) (Fig. 2A and table S2). After the first compression step to 164 GPa, the strength of the 001 maximum increases dramatically to 9.62 m.r.d. (Fig. 2B and table S2). Between 164 GPa and 185 GPa, texture changes very little (Fig. 2, B and C, and table S2). This differs from textures recorded in lower-pressure pPv analogs of Mn_2O_3 (*30*), $CaIrO_3$ (*19*, *20*–*23*), $MgGeO_3$ (*16*), and $MgSiO_3$ (*17*) but is consistent with the most recent measurements on $MgGeO_3$ pPv (*18*). These textures are stronger than textures recorded in previous experiments (*16*, *17*). In this experiment, texture strength after compression is greater than 9 m.r.d. versus ~2.5 m.r.d. in $MgSiO_3$ pPv (*17*), ~2.5 m.r.d. in $MgGeO_3$ pPv (*30*), and ~1.8 m.r.d. in $CaIrO_3$ pPv (*23*). The present results cannot be directly compared with the results of Okada *et al.* (*18*) because those measurements were qualitative.

In contrast to a previous DAC experiment on $MgSiO_3$ pPv (*17*), we observe a texture evolution with compression and conclude that the strengthening of the 001 texture is due to plastic

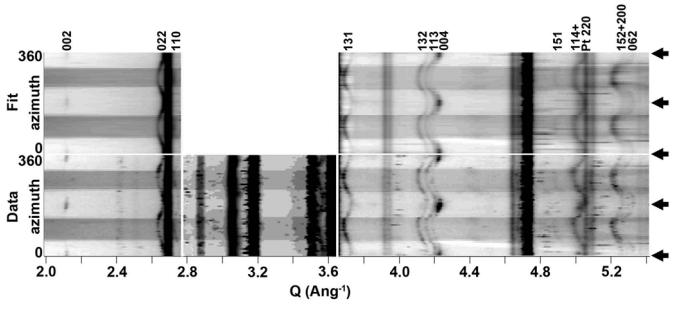


Fig. 1. "Unrolled" diffraction image of $MgSiO_3$ pPv taken in situ at 185 GPa (bottom) with the fit from Rietveld refinement (top). The region from 2.77 to 3.66 $Å^{-1}$ was excluded from Rietveld refinement due to very intense diffraction from the gasket and little signal from the sample. pPv diffraction peaks are labeled, and black arrows indicate the compression direction. Straight lines are from the gasket. The Pt 220 peak is also labeled and overlaps with the sample. Pt 111 and 200 are buried in the gasket peaks. There may also be some minor formation of PtC due to the reaction of Pt with the diamonds during laser heating (*28*). Texture is evident as systematic intensity variations along diffraction peaks. For example, pPv 004 has a strong maximum in the compression direction. Deviatoric stress can be calculated from the variation of peak position with azimuth, which is observed in the diffraction image.

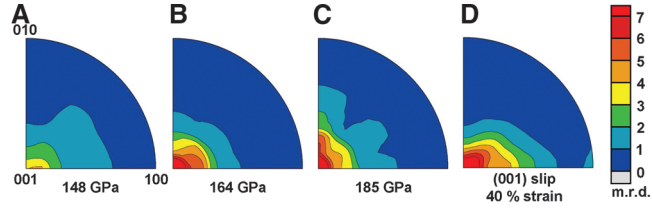


Fig. 2. Inverse pole figures of $MgSiO_3$ pPv at 148 GPa (**A**) just after transformation and at two pressure steps up to 185 GPa (**C**). Also shown for comparison is an IPF of VPSC results for dominant slip on (001) and 40% compressive strain (**D**). This provides a good match to the data after compression (**B** and C). Equal area projection and a linear scale is used. Scale bar in m.r.d., where m.r.d. = 1 is random and a higher m.r.d. number indicates stronger texture.

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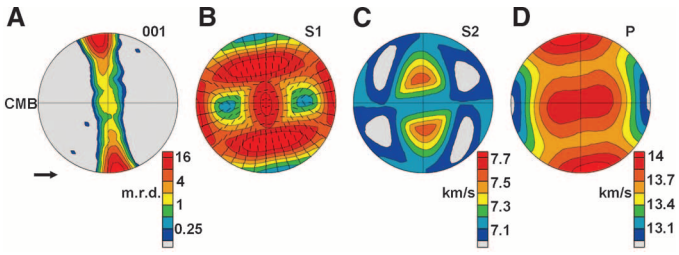


Fig. 3. Equal area projection pole figures showing texture development and anisotropic elastic properties of a pPv aggregate in a subducting slab near the core-mantle boundary using deformation mechanisms established in this study. **(A)** (001) pole figure of pPv showing a snapshot during spreading in a plane strain regime (i.e., intermediate between pure shear and simple shear). **(B)** Fast shear-wave velocities and polarization. **(C)** Slow shear-wave velocities. **(D)** *P* wave velocity surface. Flow direction indicated by arrow.

deformation. Deformation of pPv has been extensively modeled using the viscoplastic self-consistent polycrystal plasticity code [VPSC; (*31*)] (*16*, *17*, *23*). A comparison of the IPFs obtained in this study with results of VPSC models shows that the 001 texture observed here is compatible with dominant slip on (001) lattice planes and 40% compressive strain (Fig. 2D). For this model, the transformation texture obtained after conversion to pPv was used as the starting texture for the deformation simulation (Fig. 2A).

Admittedly, there are limitations to our experiments because the time scale of deformation, grain size, composition, temperature, and deviatoric stress are quite different from those expected in the *D″*. However, if we assume that slip is active on (001) planes in $MgSiO_3$ pPv at *D″* conditions and combine this information with geodynamic modeling of deformation along the *D″*, we can predict texture development in the lowermost mantle. This can, in turn, be combined with single crystal elastic constants to assess associated seismic anisotropy. For these calculations, we neglect any contribution from the second most abundant mineral in the mantle, ferropericlase, which may also play an important role in generating *D″* anisotropy (*32*).

In this context, we use information from the same two-dimensional geodynamic model applied previously (*17*, *33*) to predict texture development in a slab subducted into the *D″* zone. A tracer records the strain-temperature history and, accordingly, the texture evolution is modeled with the same polycrystal plasticity theory applied to the experiment (*31*). It is assumed that the aggregate has a random orientation distribution as it enters the *D″* about 290 km above the CMB. Based on our results, we assume dominant (001)[100] and (001)[010] slip. We chose a geodynamic tracer that records strain and temperature, which advances close to the CMB and attains large strains. Preferred orientation develops rapidly and then stabilizes; note the strong alignment of (001) lattice planes slightly inclined to the CMB (Fig. 3A). By averaging the orientation distribution and single crystal elastic properties (*34*), we calculated aggregate elastic properties and seismic wave propagation. Based on the polarization directions of the fast and slow shear-wave velocities, high shear-wave splitting is 0.55 km/s in the flow direction; fast shear waves are polarized parallel to the CMB (Fig. 3B, C). This is consistent with seismic observations of the circum-Pacific regions (*2*, *4*), where the presence of pPv is expected (*35*, *36*). The anticorrelation between fast *S* waves and *P* waves in the flow direction (Fig. 3D) is also consistent with seismic records (*37*, *38*).

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Supporting Online Material

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Materials and Methods
Figs. S1 and S2
Tables S1 and S2
References

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Genetic Restoration of the Florida Panther

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The rediscovery of remnant Florida panthers (*Puma concolor coryi*) in southern Florida swamplands prompted a program to protect and stabilize the population. In 1995, conservation managers translocated eight female pumas (*P. c. stanleyana*) from Texas to increase depleted genetic diversity, improve population numbers, and reverse indications of inbreeding depression. We have assessed the demographic, population-genetic, and biomedical consequences of this restoration experiment and show that panther numbers increased threefold, genetic heterozygosity doubled, survival and fitness measures improved, and inbreeding correlates declined significantly. Although these results are encouraging, continued habitat loss, persistent inbreeding, infectious agents, and possible habitat saturation pose new dilemmas. This intensive management program illustrates the challenges of maintaining populations of large predators worldwide.

Pumas (also called cougars, mountain lions, or panthers) are currently distributed throughout western North America and much of Central and South America (*1*). The endangered Florida panther (listed in 1967, table S1), the last surviving puma subspecies in eastern North Amer-

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ica, is restricted to shrinking habitat between the urban centers of Miami and Naples (Fig. 1). By the early 1990s, the population of ~20 to 25 adults (*2*) showed reduced levels of molecular genetic variation relative to other puma populations (*3*–*5*), which is indicative of inbreeding (*4*, *6*). This may have led to defects, including poor sperm quality and low testosterone levels (*4*, *7*), poor fecundity and recruitment (*4*, *7*), cryptorchidism [where >80% of males born from 1990 to 1992 had one or no descended testes (*4*)], a high incidence of thoracic cowlicks and kinked tails (*4*), numerous atrial septal defects (*4*, *8*), and a high load of parasites and infectious disease pathogens (*4*, *8*–*10*).

In 1995, these cumulative observations, coupled with demographic models predicting a 95% likelihood of extinction within two decades, motivated the translocation of 8 wild-caught Texas (TX) female pumas into habitat occupied by at least 22 adult canonical (last-remaining, authentic) Florida panthers (CFPs) and 4 Everglades Florida panthers (EVGs) (Fig. 1A), because historically, gene flow occurred between Texas and Florida puma populations (*11*, *12*).

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We compared data from 591 individuals sampled from 1978 to 2009 (table S2). Twenty-three informative (minimum allele frequency > 0.1) short tandem repeat (STR) loci were examined to reconstruct genetic heritage and parentage relationships; assess spatial and demographic patterns; distinguish CFP from other puma lineages; track morphological, biomedical, and life history traits as indices of fitness; and associate genetic heritage and heterozygosity with panther survival (*13*).

Pumas of diverse ancestry, time periods, and geographic origins, including wild-caught and captive animals in Florida, clustered into phylogenetic groups (Fig. 2). This analysis, combined with Bayesian population genetic results from STR genotypes that revealed nine distinct groups (fig. S1 and table S2), allowed us to explicitly infer the genetic heritage of each Florida panther (table S2, column 2; Figs. 1 and 2; and fig. S2) and to distinguish the two pre-1995 groups (CFP and EVG). Further, admixed Florida panthers (AdmFPs) were clearly identifiable, including first-generation (F_1) offspring of TX females bred by CFP or EVG males (CFPxTX-F_1 or EVGxTX-F_1) and panthers that were related to captive pumas of western U.S. origin who had escaped from enclosures on the Big Cypress Seminole Indian Reservation (SEM) from 1997 to 1999 (Figs. 1 and 2).

From 1986 to 1995, the minimum number of adult (>1.5 years old) panthers fluctuated from 24 to 32 (Fig. 3A), and genetic heritage remained relatively stable (85% CFP and 15% EVG; Fig. 1 and figs. S1 and S3). After their introduction, five of eight TX females bred (Fig. 1a) and produced 15 F_1 kittens with CFP, EVG, and at least five TX-backcross (TX-BC) offspring (figs. S3 and S4 and table S2). Twelve F_1 panthers produced offspring. From 1995 to 2008, 424 panther births were documented (81 CFP, 319 AdmFP, and 24 undetermined; 272 were observed only as neonatal kittens). These largely AdmFP were responsible for colonizing former panther range and densities increased. For example, between 1995 and 2007, the number and density of panthers in the southern Big Cypress National Preserve (BCNP) (2174 km^2) increased eightfold from 3 (0.14/100 km^2) to 25 (1.15/100 km^2) (Fig. 1).

From 1996 to 2003, numbers (N) increased by 14%/year to at least 95 adults (Fig. 3A), 26.6 kittens were produced annually (fig. S2). The effective population size (N_e) rose from 16.4 in 1995 to 32.1 by 2007, and N_e/N was 0.314 (Fig. 1) (*13*). This paralleled an increase in average individual STR heterozygosity (to 25% from 18.4% in 1993; Fig. 3B) and a decrease in the average estimated age of adults from 6.6 to 4.2 years from 1997 to 2004. Population growth slowed and average age increased gradually after 2004 (Fig. 3C).

Admixed genetic ancestry was associated with increased survival of F_1, EVG-BC, and TX-BC kittens (<1 year old) relative to purebred CFP and CFP-backcross (CFP-BC) kittens (0.518 ± 0.130 versus 0.243 ± 0.074, $P = 0.020$) (Fig. 3D). F_1 adults had significantly higher survival ($P = 0.002$) than other admixed or CFP groups (table S4), with a risk ratio (RR: relative instantaneous probability of mortality) of 0.118 (*13*). The survival of subadults and adults increased significantly with heterozygosity (RR for an increase of 0.1 = 0.643, $P = 0.011$). Interestingly, CFPs also experienced significantly higher mortality rates from intraspecific aggression than did AdmFPs (RR = 3.077, $P = 0.014$), and mortality rates from intraspecific aggression declined as heterozygosity increased (RR = 0.480, $P = 0.005$) (*13*).

Demographic differences among >1-year-old panthers from 2002 to 2004 (Fig. 3A) were evident when 23 out of 29 (23/29) (79%) CFPs alive in 2002 were lost versus 22/47 (47%) AdmFPs and when CFPs had a significantly

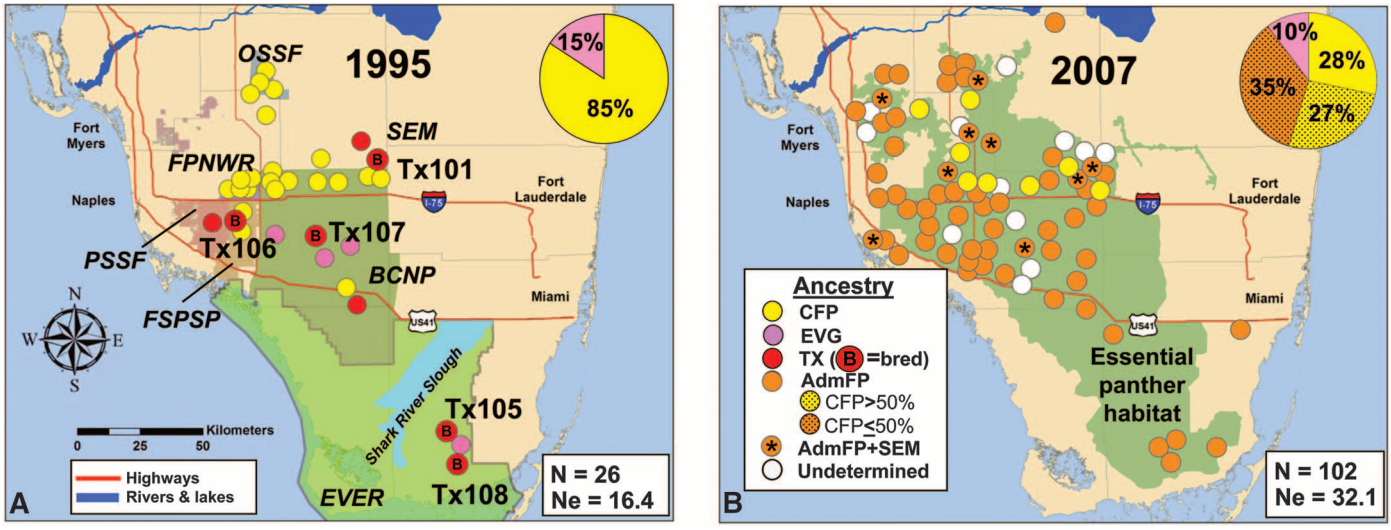


Fig. 1. (**A** and **B**) Southern Florida (1995, left; 2007, right) with locations of breeding-age Florida panthers (>1.5 years old), geographic features, number (N) alive, and effective population size (N_e). Labeled colored areas in (A) demarcate public land (*23*): Fakahatchee Strand Preserve State Park (FSPSP), Picayune Strand State Forest (PSSF), Florida Panther National Wildlife Refuge (FPNWR), BCNP, Big Cypress Seminole Indian Reservation (SEM), Okaloacoochee Slough State Forest (OSSF), and Everglades National Park (EVER) and in (B) show panther habitat. Circles are coded by ancestry: CFP, TX females (with a B if a successful breeder), EVG, AdmFP, and SEM. Pie charts illustrate the genetic heritage of the population (fig. S1 and table S2) (*13*).

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lower yearly survival (likelihood-ratio test χ^2 = 5.38, P = 0.020), dropping from 0.827 ± 0.044 (from 1997 to 2001) to 0.610 ± 0.087 [AdmFP survival declined from 0.904 ± 0.046 to 0.866 ± 0.027 (P = not significant)]. The number of documented CFP kittens went from 17 in 2002 to 5 total from 2003 to 2005, and none have been observed since (fig. S2). The CFP contribution to the AdmFP population also decreased, with only four litters (14 kittens) of CFPxAdmFP parents documented after 2004. The abrupt CFP decline (versus AdmFP increases; Fig. 3A) and differential patterns of survival and mortality are consistent with an AdmFP competitive advantage.

Panther survival is also affected by disease agents (*4*, *9*). From 2001 to 2007, 19 Florida panthers tested positive for feline leukemia virus (FeLV) antibodies, and 5 in Okaloacoochee Slough State Forest (OSSF) (Fig. 1) died with active FeLV infections (*10*). A capture and vaccination program was implemented in 2003, and no further active infections were documented after July 2004. Further, the prevalence of a puma-specific strain of feline immunodeficiency virus (FIV_{PCO}) increased 16 to 80% from 1995 to 2005. Although not explicitly implicated in clinical disease in free-ranging pumas, FIV_{PCO} infection may predispose individuals to other diseases due to low lymphocyte numbers (*14*).

A detailed population pedigree confirmed all dam/offspring inferences from field observations (278 kittens from 128 litters marked as neonates, and 51 juveniles and subadults associated with suspected dams), supported 120 of 130 sire/offspring field inferences, and identified an additional 174 probable parents (48 dams and 126 sires). At least one parent was assigned to 422 individuals: 74 different dams and 49 sires to 397 and 298 offspring, respectively (table S2 and figs. S3 and S4). The estimated relative genetic contribution of the TX females to the descendant population varied widely (TX101 = 0.20, TX105 = 0.01, TX106 = 0.06, TX107 = 0.10, and TX108 = 0.04).

Shrinking and fragmented populations are at high risk for inbreeding depression (*15*, *16*) and local extinction (*17*) through demographic and stochastic events (*18*). These influences probably caused the precipitous decline in Florida panther N_e from 1900 to 1980 (*19*). The stated goal of the Florida panther genetic restoration plan was to improve population size and viability by increasing genetic variability without losing unique local adaptations. By several measures, this experiment was successful. Most notably, after the introduction of Texas females, the population tripled, with a parallel significant reduction in the incidence of several phenotypic characters historically associated with inbreeding depression (Table 1). Additionally, admixed panthers exhibited behaviors that might be associated with higher fitness, as evidenced by increased escape behavior from high trees during capture (RR = 2.0, P = 0.001, fig. S5).

In addition to genetic restoration, enhancement in panther numbers was probably facilitated by action initiated in the late 1980s by federal, state, and private groups to mitigate panther declines and facilitate natural recovery. This included buttressing legal protection under the Endangered Species Act (*20*), acquiring and protecting >120,000 ha of occupied panther habitat, altering prey management (*21*), and constructing highway underpasses to reduce mortality from vehicle strikes (*22*). In

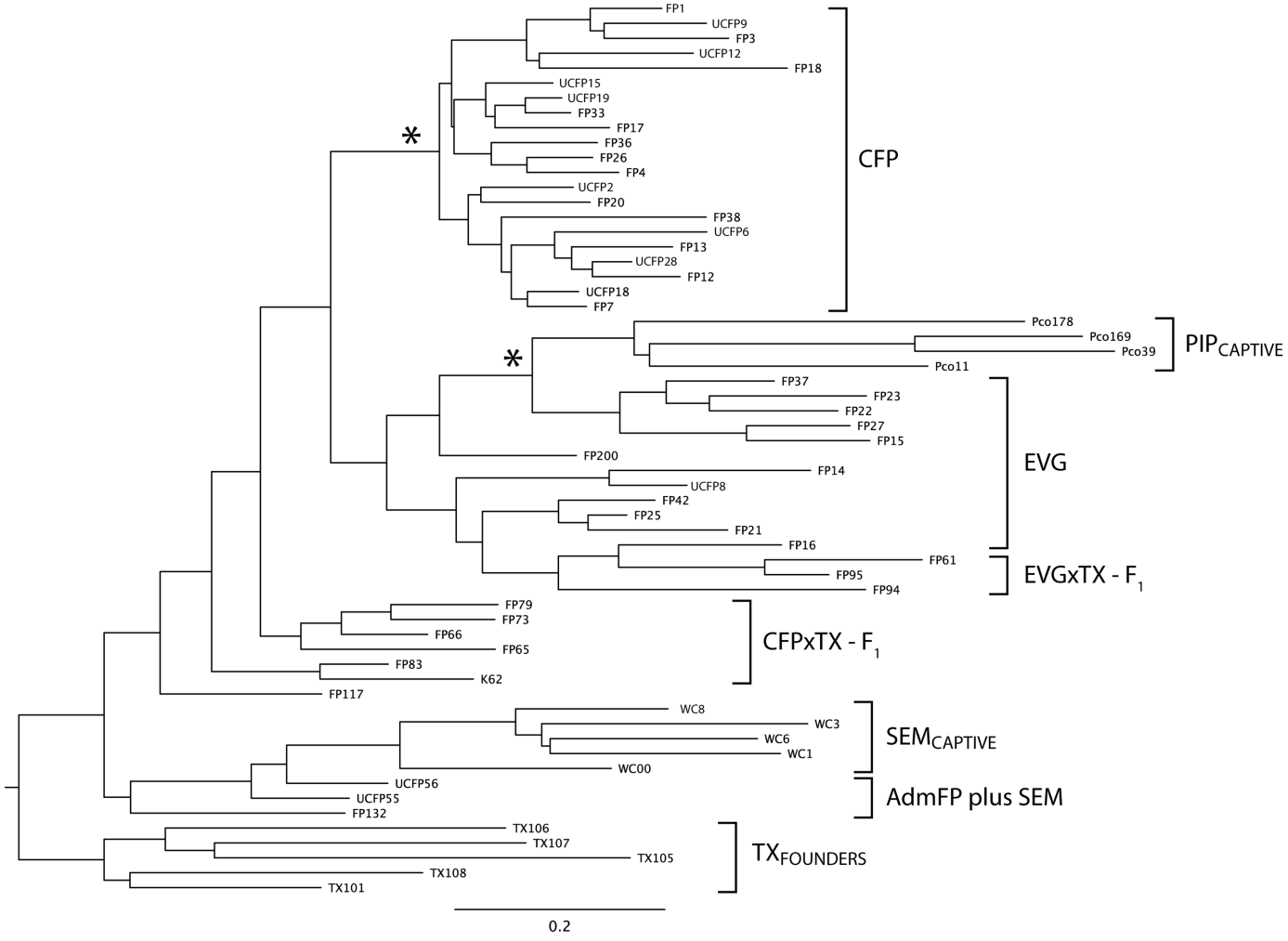


Fig. 2. Neighbor-joining tree of composite STR genotypes portraying genetic relationships among pre-1995 founding CFPs, introduced TX females, EVG and F_1 admixed Florida panthers (CFPxTX-F_1 and EVGxTX-F_1). Captive Piper panthers ($PIP_{captive}$) are related to present-day EVGs through individuals released into the Everglades between 1957 and 1967 (*3*, *4*), and SEMs contributed unintended gene flow. Asterisks mark nodes with >80% bootstrap support (*13*).

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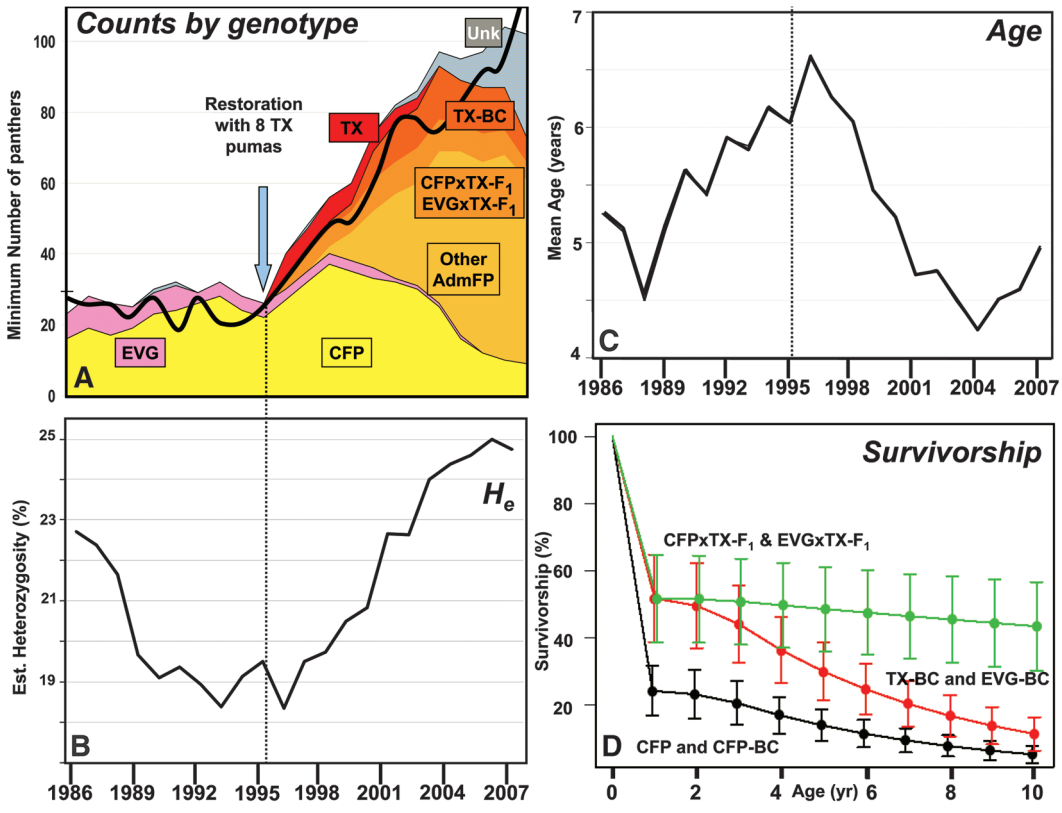


Fig. 3. (**A**) Minimum annual subadult and adult panther population size and inferred genetic heritage from 1986 to 2007. CFPs are yellow, EVGs pink, TXs red, CFPxTX-F_1s and EVGxTX-F_1s orange, TX-BCs and other AdmFPs shades of orange, and genetically uncharacterized individuals (Unk) gray (*13*). The black line is an independent minimum-count estimate from surveys of tracks, spoor, and other field evidence (*2*). (**B**) Mean yearly adult multilocus heterozygosity. (**C**) Yearly mean age of adults. (**D**) Projected survivorship (probability of surviving to an age) curves for female Florida panthers of different genetic heritages with standard error bars (*13*). Male trends are similar (table S4).

Table 1. Estimates of molecular genetic variation and prevalence of physiological and morphologic traits in Florida panthers of different genetic heritages (*13*) (see table S3). NA, not available.

			Crytorchidism**			Prevalence of**			
Heritage group	No.	Average* heterozygosity	Avg. no. of descended testicles	Prevalence in males	Normal sperm (%) Ejaculate (EJ) Gamete rescue (GR) (No. of males)	Atrial septal defects	Kinked tails	Cowlick on thorax	Percent of** individuals with ≥1 abnormal trait
CFP	116	0.167 ± 0.005^{A}	1.3 ± 0.07^{A}	0.66 ± 0.06^{A}	EJ 5.4 ± 0.7^{C} (15) GR 10.1 ± 1.9^{N} (13)	0.17 ± 0.05^{A}	0.90 ± 0.03^{A}	0.81 ± 0.04^{A}	70.3 ± 2.5^{A}
EVG	17	0.282 ± 0.022^{B,D}	2.0 ± 0^{B}	0^{B}	9.5 ± 0.6^{B} (5)	0^{A}	0.31 ± 0.12^{B}	0.29 ± 0.11^{B}	22.5 ± 7.1^{B}
TX	5	0.318 ± 0.02^{B,D}	2.0 ± 0^{B}§	0^{B}§	14.0 ± 3.5^{A,B}§ (9)	0^{A}	0^{B}	0^{B}	0^{B}
All AdmFP	143	0.244 ± 0.006^{B}	1.9 ± 0.0^{B}	0.10 ± 0.035^{B}	(See below)	0.08 ± 0.030^{A}	0.25 ± 0.037^{B}	0.27 ± 0.039^{B}	19.5 ± 2.1^{B}
AdmFP groups									
TX-F_1	10	0.336 ± 0.01^{B,}	2.0 ± 0	0^{B}	EJ 20.5 ± 4.5^{A} (2)	0^{A}	0^{B}	0.38 ± 0.18^{B}	14.6 ± 7.3^{B}
TX-BC	18	0.273 ± 0.016^{B,D}	2.0 ± 0^{B}	0^{B}	NA	0.14 ± 0.14^{A}	0^{B}	0.06 ± 0.06^{B}	3.2 ± 2.2^{B}
Other AdmFP	52	0.251 ± 0.008^{C,D}	1.94 ± 0.04^{B}	0.06 ± 0.042^{B}	EJ 7.0 ± 6.0 (2) GR 16.6 ± 3.2^{P} (10)	0.065 ± 0.045^{A}	0.160 ± 0.05^{B}	0.22 ± 0.0^{B}	14.4 ± 3.2^{B}
Non–TX-BC	63	0.216 ± 0.008^{C}	1.8 ± 0.06^{B}	0.17 ± 0.06^{B}	NA	0.08 ± 0.04^{A}	0.43 ± 0.070^{B}	0.36 ± 0.07^{B}	29.4 ± 3.3^{B}

*t test and **Fisher's exact test: Column values (mean ± SE) with different superscript letters (A to D; except GR sperm, which is N and P) are significantly different ($P < 0.05$). §From (*7*).

spite of improvements, ongoing density-dependent factors (related to limited and decreasing habitat availability) and stochastic events will continue to regulate population growth, requiring continued commitments to identify and maintain additional quality habitat to preserve Florida panther evolutionary potential for the long term.

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24. We dedicate this study to the memory of Ulysses Seal and Ernst Mayr, important heroes in the conservation struggle of the Florida panther. Funded by the Florida Fish and Wildlife Conservation Commission (FWC) via purchases of Florida panther license plates. Other major funding for the field work was provided by Everglades National Park (EVER), the BCNP, and federal funds from the U.S. Fish and Wildlife Service (especially in the early years) as well as from the National Cancer Institute, National Institutes of Health (NIH), under contract number N01-CO-12400. This research was supported in part by the Intramural Research Program of NIH, National Cancer Institute, Center for Cancer Research, and the Florida Panther Research and Management Trust Fund. All panther captures, sampling, and radio collaring were authorized by U.S. Fish and Wildlife Service Endangered Species Permits TE01553-3 (FWC) and TE146761-1 (BCNP). We thank many individuals for their help in this project, who are named in SOM reference S25.

Supporting Online Material

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Materials and Methods
Figs. S1 to S5
Tables S1 to S6
References

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Parasympathetic Innervation Maintains Epithelial Progenitor Cells During Salivary Organogenesis

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The maintenance of a progenitor cell population as a reservoir of undifferentiated cells is required for organ development and regeneration. However, the mechanisms by which epithelial progenitor cells are maintained during organogenesis are poorly understood. We report that removal of the parasympathetic ganglion in mouse explant organ culture decreased the number and morphogenesis of keratin 5–positive epithelial progenitor cells. These effects were rescued with an acetylcholine analog. We demonstrate that acetylcholine signaling, via the muscarinic M1 receptor and epidermal growth factor receptor, increased epithelial morphogenesis and proliferation of the keratin 5–positive progenitor cells. Parasympathetic innervation maintained the epithelial progenitor cell population in an undifferentiated state, which was required for organogenesis. This mechanism for epithelial progenitor cell maintenance may be targeted for organ repair or regeneration.

Organogenesis involves the coordinated growth of epithelium, mesenchyme, nerves, and blood vessels, which use common sets of genes, guidance cues, and growth factor–signaling pathways (*1*–*5*). Research on epithelial organogenesis has focused on epithelial-mesenchymal and endothelial-epithelial cell interactions. However, the function of the peripheral nervous system during epithelial organogenesis is less clear.

Pavlov's seminal experiments on dogs demonstrated that neuronal input controls salivary gland function (*6*), and more recent work showed that parasympathetic innervation of salivary glands is essential for regeneration after injury (*7*). Because parasympathetic innervation occurs in parallel with salivary gland development (*8*), we hypothesized that parasympathetic innervation is required for epithelial progenitor cell function during organogenesis.

To test this hypothesis, we used mouse embryonic submandibular gland (SMG) explant culture and mechanically removed the parasympathetic submandibular ganglion (PSG) before the gland developed (*9*). SMG development begins at embryonic day 11 (E11), when the oral epithelium invaginates into neural crest–derived mesenchyme (*10*). The neuronal bodies of the PSG condense around the epithelium at E12 (fig. S1A) and could be separated from epithelium and mesenchyme in explant culture. When the separated tissues were recombined in culture, the growth of the SMG epithelium was reduced, with a significant decrease in the number of end buds in the absence of the PSG (Fig. 1, A and B, and fig. S2A). The PSG axons have abundant varicosities (fig. S2B, box) that contain the neurotransmitter acetylcholine (ACh) (*8*), and express the ACh synthetic enzyme (*Chat*) (fig. S1, B and C). ACh activates epithelial muscarinic (M) receptors, and M1 (*Chrm1*) is the major muscarinic receptor in the embryonic SMG epithelium (fig. S1, B and C), whereas M1 and M3 (*Chrm3*) stimulate saliva secretion in the adult (*7*). Alternatively, we perturbed ACh/M1 signaling using the chemical inhibitors, 4-DAMP (DAMP; *N*-2-chloroethyl-4-piperidinyl diphenylacetate), an irreversible M1/M3 inhibitor (Fig. 1C); atropine, a competitive muscarinic antagonist (fig. S2D); beta-bungarotoxin (Btx), which depletes neuronal ACh stores (fig. S2E); and small interfering RNA (siRNA) to M1 (*Chrm1*) (fig. S2, H to I). All treatments reduced the number of end buds (fig. S2, C to I). In contrast, inhibition of α_2-adrenergic receptors with idazoxan had no effect (fig. S2F). These experiments demonstrate that epithelial morphogenesis requires PSG, ACh, and M1 activity.

Epithelial morphogenesis may also depend on the size of the epithelial progenitor pool and growth factor–mediated proliferation (*11*). To distinguish between these two possibilities, we mea-

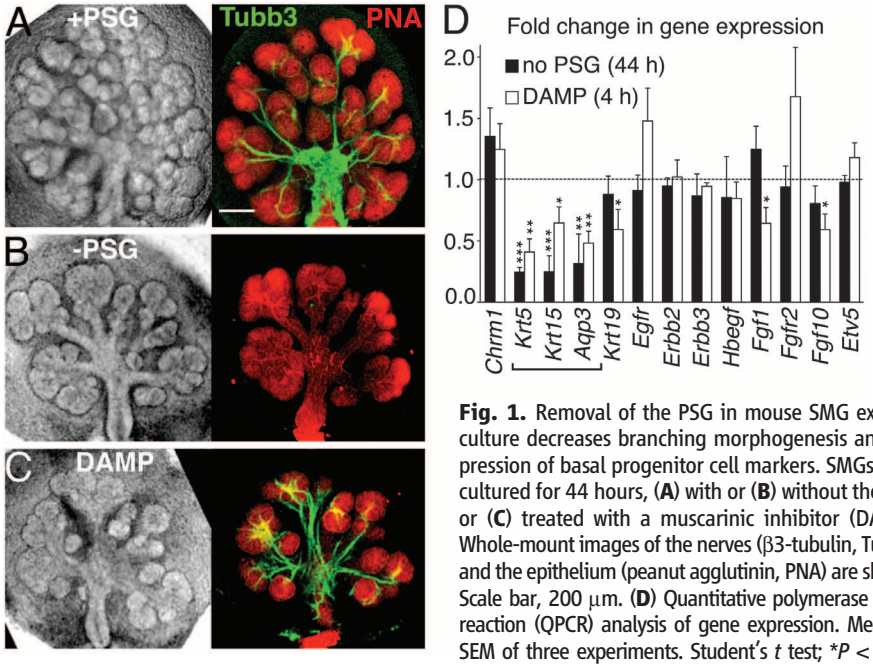


Fig. 1. Removal of the PSG in mouse SMG explant culture decreases branching morphogenesis and expression of basal progenitor cell markers. SMGs were cultured for 44 hours, (**A**) with or (**B**) without the PSG or (**C**) treated with a muscarinic inhibitor (DAMP). Whole-mount images of the nerves (β3-tubulin, Tubb3) and the epithelium (peanut agglutinin, PNA) are shown. Scale bar, 200 μm. (**D**) Quantitative polymerase chain reaction (QPCR) analysis of gene expression. Means ± SEM of three experiments. Student's *t* test; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

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sured expression of epithelial progenitor markers and growth factor signaling pathways present during SMG development. We found that removal of the PSG reduced gene expression of the epithelial progenitor cell markers cytokeratins-5 (*Krt5*) and -15 (*Krt15*), as well as aquaporin 3 (*Aqp3*) (Fig. 1D), and did not affect genes involved in fibroblast growth factor (FGF) and epidermal growth factor (EGF) signaling. Cytokeratin-5 protein (referred to as K5) is a basal epithelial cell marker in adult salivary glands (*12*–*14*). Developing SMGs contain 9.6 ± 1.3% of K5+ cells by fluorescence-activated cell sorting (FACS) analysis, which are present in the end buds and ducts (fig. S3, A to C). K5+ cells are progenitor cells in the trachea (*15*) and prostate (*16*), and we confirmed that they are progenitor cells in the SMG by lineage tracing analysis (fig. S3, D to H). Cytokeratin-15 protein (K15) can physically pair with K5 (*17*); and Aqp3 is an epithelial progenitor cell marker in the lung (*15*, *18*). *Krt5*, *Krt15*, and *Aqp3* were down-regulated in intact SMGs after only 4 hours of DAMP treatment (Fig. 1D), which indicates that they are regulated by ACh/M1 signaling. Taken together, these data support our hypothesis that the PSG neurons modulate epithelial morphogenesis by affecting epithelial progenitor cells via ACh/M1 signaling.

To investigate how ACh directly influences the epithelium, we cultured isolated SMG epithelia in a three-dimensional extracellular matrix with FGF10 (*19*). We hypothesized that carbachol (CCh), an ACh analog, would increase epithelial morphogenesis and proliferation by increasing the K5+ progenitor cell population. Because ACh/M1 signaling transactivates epidermal growth factor receptor (EGFR) by matrix metalloproteinase (MMP)–mediated release of heparin-binding (HB)–EGF in prostate epithelium (*20*), we predicted that (i) HBEGF would increase K5+ cell proliferation, and (ii) an EGFR antagonist (PD168393, referred to here as PD) would inhibit CCh-induced K5+ cell proliferation. As expected, CCh and HBEGF increased epithelial morphogenesis, proliferation, and K5 staining, in an EGFR-dependent manner (Fig. 2, A to F, and fig. S4, A to B). Furthermore, CCh increased EGFR protein expression (fig. S4, C to F), and CCh-mediated morphogenesis was inhibited by PD (fig. S4G), which suggests that muscarinic-induced morphogenesis requires endogenous EGFR activity. When CCh and HBEGF were combined, they had a greater-than-additive effect on morphogenesis and proliferation, which were both inhibited by PD (Fig. 2, D to F). However, the combination did not have an additive effect on K5 staining. Therefore, CCh and HBEGF operate in the same pathway to maintain K5, and they increase proliferation of cells that do not express K5.

To investigate this further, we analyzed differentiation of the K5+ cell population. K5+ progenitor cell differentiation occurs in a manner similar to that described for the prostate (*16*); as the basal K5+K19– progenitor cells differentiate toward the developing lumen, they coexpress K19 (K5+K19+), and as differentiation proceeds, they lose K5 (K5–K19+) (Fig. 2G and fig. S5). Thus, the K5+ cell population (Fig. 2, A to F) includes both basal K5+K19– cells and K5+K19+ cells. To identify differences in the response to either CCh or HBEGF, we counted the proliferating cells (5-ethynyl-2′-deoxyuridine or EdU+) that were K5+K19– (green), K5+K19+ (yellow), or K5–K19+ (red) (Fig. 2H and fig. S5). An important finding was that CCh doubled the percentage of proliferating basal K5+K19– progenitor cells from 20 to 40% of the EdU+ cells. CCh also increased K5+K19+ cell proliferation, which suggested that these cells are still responsive to muscarinic activation. HBEGF increased the proliferation of both the K5+K19+ and K5–K19+ cells, which suggests that HBEGF promotes differentiation along the K19+ lineage. The combination of CCh and HBEGF resulted in similar amounts of proliferation of K5+K19–, K5+K19+, and K5–K19+ cells (Fig. 2F). Finally, CCh- and HBEGF-mediated proliferation of the K5+ and K19+ cells was completely inhibited by PD (Fig. 2H), although proliferating cells that were not K5+ or K19+ were still present (fig. S5). Taken together, these data suggest that CCh/M1 signaling maintains the K5+ progenitor cell population in an EGFR-dependent manner, and that HBEGF/EGFR increases differentiation of the K19+ cell lineage.

We confirmed these findings in the intact SMG, by using a loss-of-function approach treating SMGs with DAMP or PD. We hypothesized that DAMP treatment would inhibit K5+ cell proliferation and result in fewer K5+ cells. We used FACS analysis of the E-cadherin+ epithelial population, measuring the number of cells expressing K5, K19, and Ki67 for proliferating cells (fig. S6, A to D). As expected, the total K5+ cell population was reduced by both DAMP and PD treatment. The reduction in K5+ cell number was not due to an increase in apoptosis (fig. S7). Note that both PD and DAMP completely inhibit proliferation of the K5+K19– basal progenitor cells. In sum, the inhibition of M1 and EGFR signaling in the intact SMG reduces the number of K5+K19– progenitor cells by inhibiting their proliferation. These data demonstrate that the maintenance of K5+ cells in an undifferentiated state (K5+K19–) in the intact SMG is dependent on M1 and EGFR signaling.

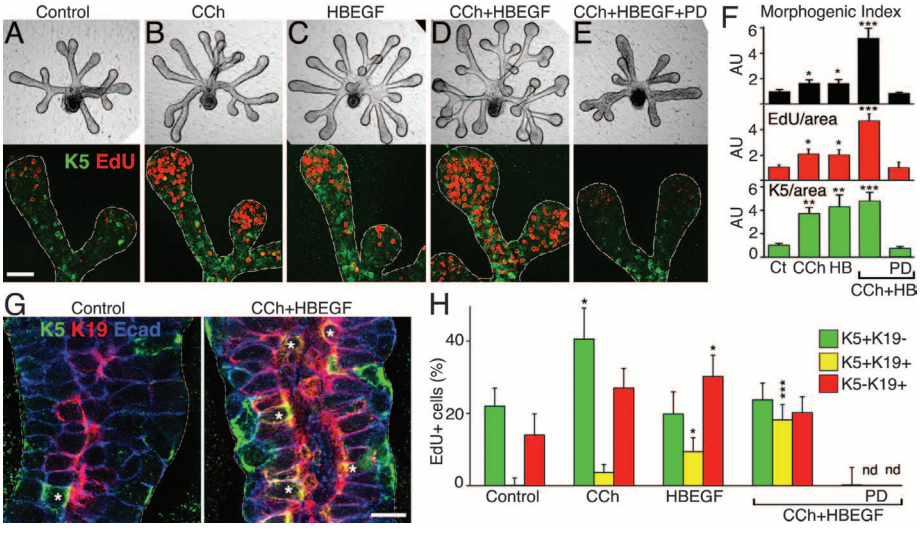


Fig. 2. Activation of muscarinic receptors maintains K5+K19– progenitor cells in an EGFR-dependent manner. Epithelia were cultured (**A**) in control media, or with (**B**) CCh, (**C**) HBEGF, (**D**) CCh+HBEGF, or (**E**) CCh+HBEGF+PD. Immunostaining of proliferation (EdU) and K5+ cells is shown. Images are 3 μm confocal sections; scale bar, 50 μm. (**F**) Quantification of epithelial morphogenesis, proliferation, and K5 protein. AU, arbitrary units × 100. (**G**) Epithelia were immunostained for K5, K19, and E-cadherin. Yellow cells expressing both K5 and K19 are marked by white asterisks. Images are 2 μm confocal sections; scale bar, 10 μm. (**H**) Quantification of the number of proliferating cells; see fig. S5 for images. Means ± SEM of three experiments. Analysis of variance (ANOVA) with post hoc Dunnett's test; **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

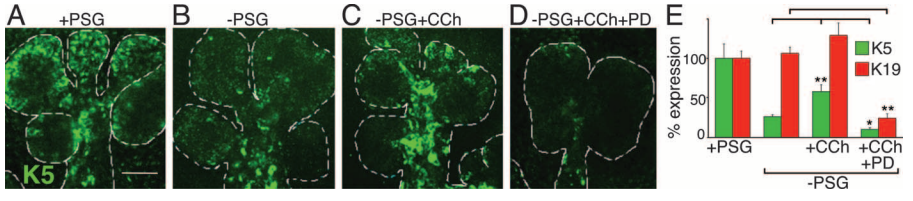


Fig. 3. CCh rescues branching morphogenesis and K5+ progenitor cells in an EGFR-dependent manner. SMG explants, recombined (**A**) with or (**B** to **D**) without the PSG, were cultured with (**C**) CCh or (**D**) CCh+PD. Images are single confocal sections (2 μm); scale bar, 50 μm. (**E**) Quantification of K5 and K19 fluorescence. Means ± SEM of three experiments. ANOVA with post hoc Dunnett's test; **P* < 0.05, ***P* < 0.01.

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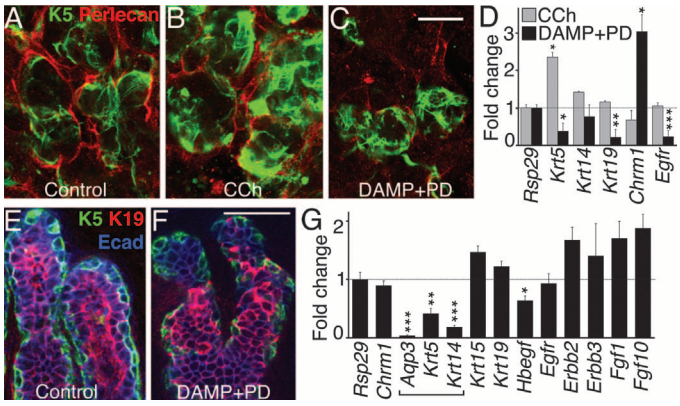


Fig. 4. Muscarinic receptor and EGFR signaling controls K5+ progenitor cell maintenance in the adult SMG and developing prostate. Denervated adult SMGs (**A**), cultured with (**B**) CCh or (**C**) DAMP+PD, were immunostained for K5 and perlecan, and (**D**) analyzed by QPCR. Scale bar, 20 μm, see fig. S10 for quantification. Ventral prostates from P6 mice were cultured (**E**) without or (**F**) with DAMP+PD and immunostained for K5, K19, and E-cadherin, and (**G**) gene expression was analyzed by QPCR. Scale bars, 50 μm. Means ± SEM of three experiments. Student's *t* test; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

These findings suggest that K5+ progenitor cells express both M1 and EGFR. Using immunostaining and FACS analysis, we demonstrated that the majority of K5+ cells (68%) expressed both EGFR and M1 (fig. S8, A to H), which suggests that the effects of CCh and HBEGF are cell-autonomous. Furthermore, the combination of HBEGF and CCh increased the amount of both M1 and EGFR in the epithelium (fig. S8, J to N), which shows that positive feedback increases receptor expression.

We then predicted that CCh would rescue epithelial morphogenesis and K5 expression in the PSG-free SMG explants in an EGFR-dependent manner. As expected, treatment of SMG with PD, or removal of the PSG, decreased the number of end buds and the amount of K5 staining compared with the control (Fig. 3, A to E, and fig. S9). Treatment of the PSG-free explants with CCh increased the number of end buds and K5 expression, which were inhibited by PD (Fig. 3, C to E). Furthermore, PD reduced K19 expression in the absence of the PSG, which demonstrates that endogenous HBEGF maintains the K19+ cells. HBEGF increases SMG morphogenesis by inducing membrane type 2–MMP and FGF receptor (FGFR) expression (*21*). Thus, addition of PD to PSG-free explants reduces the EGFR-dependent morphogenesis, and the remaining growth is likely FGFR2b-dependent (*10*, *19*).

Our data demonstrate that parasympathetic innervation maintains K5+ progenitor cells during epithelial organogenesis. A therapeutic implication is that postnatal epithelial regeneration of salivary glands may require muscarinic stimulation of the K5+ progenitor cells. Indeed, culture of denervated lobules of adult SMGs with CCh increased K5 and *Krt5* expression, which were both reduced with a combination of DAMP and PD (Fig. 4, A to D, and fig. S10A). In addition, K5+ cells increase during regeneration of adult SMGs after duct ligation when the innervation to the gland is intact (*22*). Furthermore, other organs that contain K5+ progenitor cells, such as the prostate (*16*), the skin and its appendages (*23*), the airway epithelium and trachea (*15*), and taste buds (*24*), are innervated by the peripheral parasympathetic nervous system (*25*) during development. We analyzed the developing ventral prostates of P6 mice, which contain basal K5 cells and innervation from the pelvic ganglion neurons (fig. S10B). The addition of DAMP and PD to prostate organ culture also decreased K5 expression (Fig. 4, E to F, and fig. S10C), and reduced *Krt5*, *Krt14*, and *Aqp3* (Fig. 4G). Thus, we report a mechanism by which K5+ epithelial progenitor cells are maintained during development, which has implications for understanding how tissue-specific epithelial progenitor cells could be targeted for organ repair or regeneration.

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Supporting Online Material

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Materials and Methods
Figs. S1 to S10
References

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Olfactory Plasticity Is Regulated by Pheromonal Signaling in *Caenorhabditis elegans*

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Population density–dependent dispersal is a well-characterized strategy of animal behavior in which dispersal rate increases when population density is higher. *Caenorhabditis elegans* shows positive chemotaxis to a set of odorants, but the chemotaxis switches from attraction to dispersal after prolonged exposure to the odorants. We show here that this plasticity of olfactory behavior is dependent on population density and that this regulation is mediated by pheromonal signaling. We show that a peptide, suppressor of NEP-2 (SNET-1), negatively regulates olfactory plasticity and that its expression is down-regulated by the pheromone. NEP-2, a homolog of the extracellular peptidase neprilysin, antagonizes SNET-1, and this function is essential for olfactory plasticity. These results suggest that population density information is transmitted through the external pheromone and endogenous peptide signaling to modulate chemotactic behavior.

Caenorhabditis elegans is attracted to a series of odorants, such as benzaldehyde (bz) (*1*). However, after extended exposure to the odor in the absence of food, worms stop approaching the odorant and disperse from it—a behavioral plasticity called olfactory adaptation or food-odor associative learning (hereafter called olfactory plasticity) (*2*–*4*). The odorant

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probably serves as a signal of remote food, and the naïve chemotaxis to the odorant is probably beneficial for *C. elegans* in nature, but if worms fail to reach food after a certain period of odor sensation they no longer approach the odorant. For the best chance of survival, animals may have to optimize the extent of this behavioral switching by assessing environmental conditions, including the population density of the species, which can critically affect the survival strategy (*5*–*7*). Over the course of our olfactory plasticity assays (fig. S1), we noticed that the extent of olfactory plasticity shows positive correlation with the density of animals on the cultivation plates (Fig. 1A).

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Population density is known to regulate the developmental decision of dauer larva formation, and for this regulation the population density information is transmitted by the dauer pheromone, which is continuously secreted by the animals (*8*). Mutants of *daf-22*, which encodes an enzyme in the pheromone biosynthesis pathway (*9*), showed a defect in olfactory plasticity (Fig. 1B and figs. S2 and S3). The chemical nature of dauer pheromones was recently described as a set of sugar derivatives called ascarosides (*10*–*13*). Addition of either crude pheromone extract or individual synthetic pheromones restored olfactory plasticity to the *daf-22* mutants (Fig. 1B and fig. S4). Long-term incubation with the pheromone (more than 24 hours) before the assay is needed to exert full effect of the pheromone (fig. S5). We therefore conclude that population density information is transmitted via the dauer pheromone ascarosides, which enhance olfactory plasticity.

To learn how olfactory plasticity is regulated, we screened for mutants defective in the plasticity of chemotaxis to bz (*14*, *15*). Genetic fine-mapping and rescue experiments revealed that one of the mutants, *pe356*, had a mutation in a splicing acceptor of the *nep-2* gene, which encodes a *C. elegans* homolog of the mammalian peptidase family neprilysin (Fig. 2, A to C, and figs. S2, S3, S6A, and S8). Neprilysin is a type II transmembrane protein with a zinc metalloprotease activity in the C-terminal ectodomain and is known to degrade extracellular peptides (*16*, *17*). A deletion mutant of *nep-2*, *pe379*, also showed a severe defect in olfactory plasticity (Fig. 2, A and B). Metalloprotease activity is probably essential for regulation of olfactory plasticity because a mu-

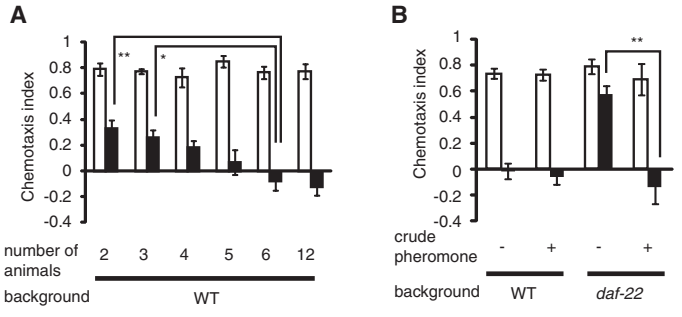


Fig. 1. Olfactory plasticity is regulated by population density and dauer pheromone. (**A**) Two to 12 parental adults per plate were put on the culture plates, and the progeny was tested for olfactory plasticity ($n \geq 6$ assays). (**B**) Olfactory plasticity of *daf-22* mutants was tested with or without adding 0.03 unit/ml of crude dauer pheromone to the culture plates ($n \geq 6$ assays). Open bars and filled bars represent mock preexposed condition and bz preexposed condition, respectively. $^{*}P < 0.01$, $^{**}P < 0.001$ for all figures; (B) Student's *t* test and (A) Dunnett's test were used for multiple comparisons. Error bars represent SEM in all figures.

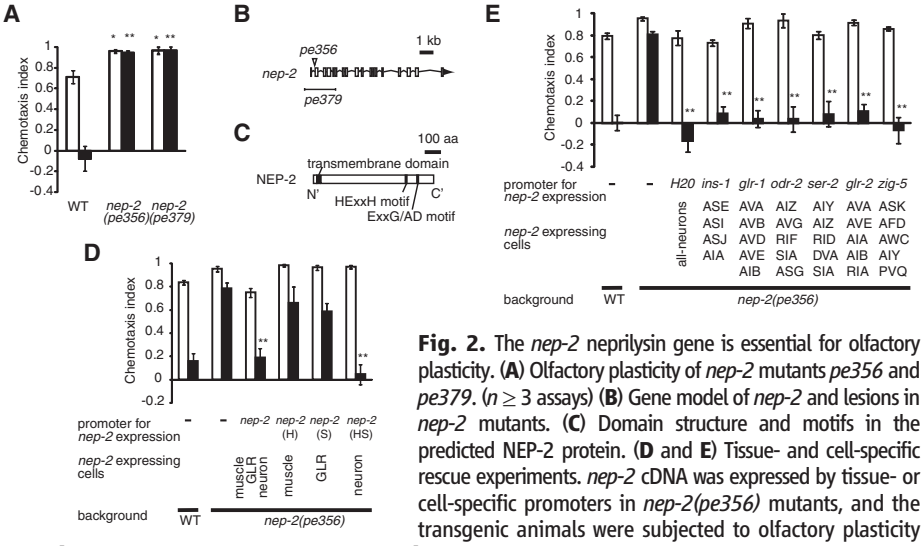


Fig. 2. The *nep-2* neprilysin gene is essential for olfactory plasticity. (**A**) Olfactory plasticity of *nep-2* mutants *pe356* and *pe379*. ($n \geq 3$ assays) (**B**) Gene model of *nep-2* and lesions in *nep-2* mutants. (**C**) Domain structure and motifs in the predicted NEP-2 protein. (**D** and **E**) Tissue- and cell-specific rescue experiments. *nep-2* cDNA was expressed by tissue- or cell-specific promoters in *nep-2(pe356)* mutants, and the transgenic animals were subjected to olfactory plasticity assay [(D) $n \geq 4$ assays and (E) $n \geq 5$ assays]. Open bars and filled bars represent mock preexposed conditions and bz preexposed conditions, respectively. Dunnett's test was used for multiple comparisons.

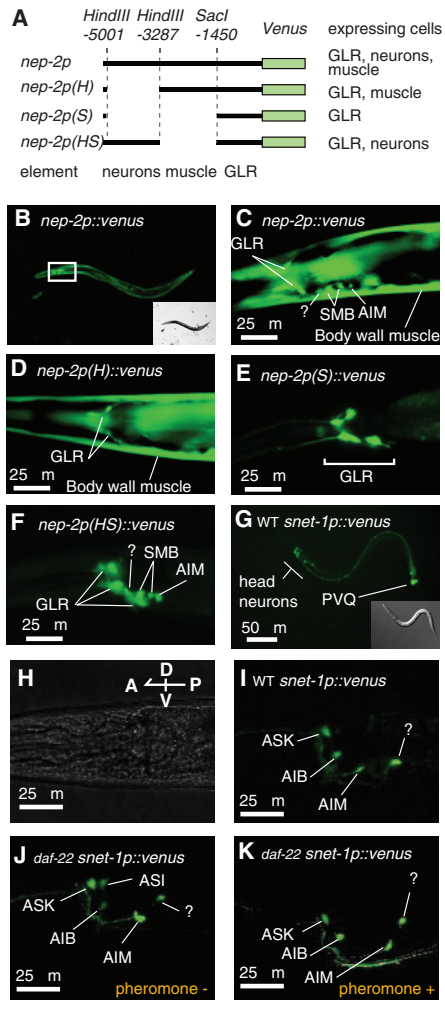


Fig. 3. Expression patterns of *nep-2* and *snet-1*. (**A**) Structure of the truncated *nep-2* promoters, *nep-2p*, *nep-2p(H)*, *nep-2p(S)*, and *nep-2p(HS)*, used for the expression of Venus reporter. (**B** to **F**) Expression pattern of Venus reporter driven by *nep-2p*, *nep-2p(H)*, *nep-2p(S)*, or *nep-2p(HS)*. Bright-field image of (B) is shown as an inset. (C) is a magnified image of the depicted region of (B). (D) to (F) show the same head region as shown in (C). (**G** to **I**) Expression pattern of the Venus reporter driven by *snet-1p*. Bright-field image of (G) is shown as an inset. (H) shows a bright-field image of the head region observed in (I). (**J** and **K**) Expression pattern of Venus reporter driven by *snet-1p* in the *daf-22* background with or without addition of 0.1 unit per milliliter of dauer pheromone to the culture plate. Question marks indicate an unidentified pair of lateral neurons.

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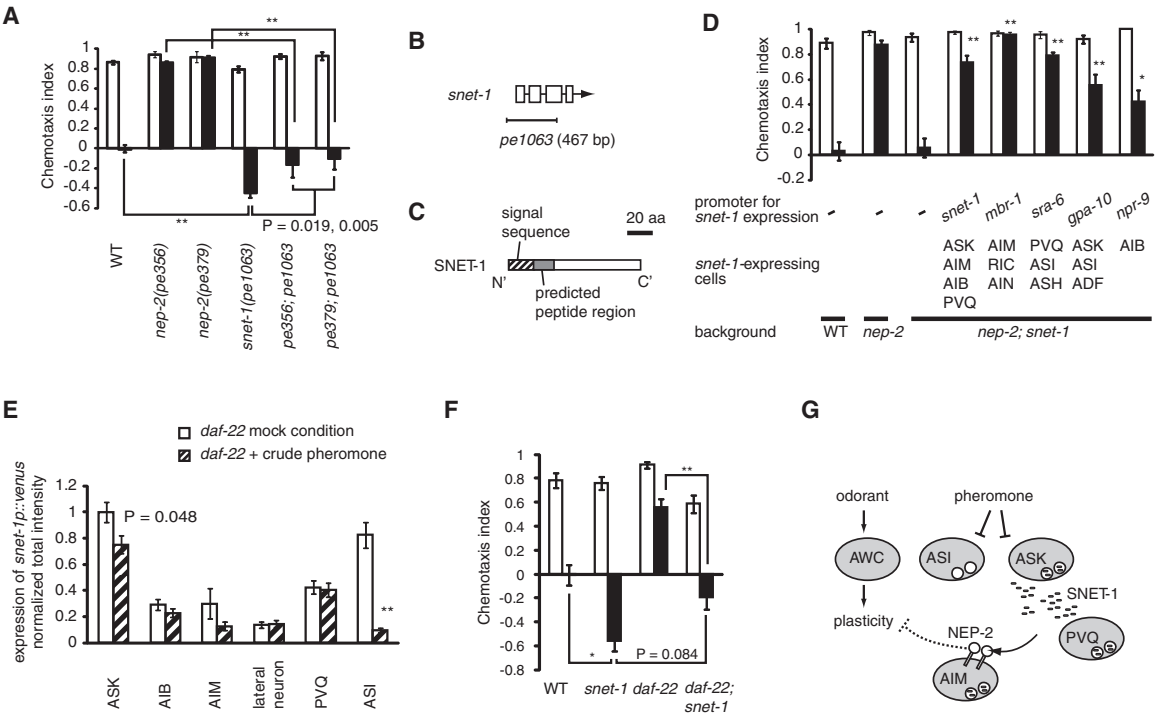


Fig. 4. *snet-1* encodes a peptide and regulates olfactory plasticity. (**A**) Olfactory plasticity of *nep-2*, *snet-1*, and *nep-2;snet-1* mutants ($n \geq 5$ assays). (**B**) Gene model of *snet-1* and the *pe1063* mutation. (**C**) Domain structure in the predicted SNET-1 protein. (**D**) *snet-1* cDNA was expressed in the *nep-2(pe379);snet-1(pe1063)* double-mutant background by cell-specific promoters, and the transgenic animals were subjected to olfactory plasticity assay ($n \geq 5$ assays). (**E**) Relative levels of *snet-1p::venus* fluorescence in adult *daf-22* mutant animals with or without 0.1 unit per milliliter of crude pheromone in the culture plates ($n = 80$ animals for ASK and $n = 40$ animals for other neurons). (**F**) Olfactory plasticity of the *daf-22(m130);snet-1(pe1063)* double mutant ($n \geq 5$ assays). (**G**) A possible role for NEP-2 and SNET-1 in pheromone-dependent regulation of olfactory plasticity. In (A), (D), and (F), open bars and filled bars represent mock preexposed conditions and bz preexposed conditions, respectively. [(A) and (F)] Tukey's test, (D) Dunnet's test, and (E) Student's *t* test with Bonferroni correction were used for multiple comparisons.

tant form of *nep-2*, *nep-2(FExxF)*, in which zinc-binding amino acids at the active center were mutated (*18*), failed to rescue the mutant phenotype (fig. S7).

Observation of the fluorescent reporter Venus fused to the *nep-2* promoter suggested that *nep-2* is mainly expressed in muscles, glia-like cells (GLRs), and several classes of neurons (Fig. 3, B and C). Expression in each of these tissues is driven by separate cis-acting elements in the promoter region (Fig. 3, A and D to F). The translational fusion protein NEP-2::Venus was localized to the cell surface in both muscles and neurons (fig. S7, D to H). Tissue- and cell-specific rescue experiments show that expression of *nep-2* in the nervous system is important for olfactory plasticity (Fig. 2D) and that *nep-2* can be functional when expressed in a variety of neurons (Fig. 2E). These observations are consistent with NEP-2 acting as an ecto-peptidase that degrades paracrine or endocrine peptides so that accumulation of the peptide in extracellular space in the *nep-2* mutant causes inhibition of olfactory plasticity.

To identify neuropeptides that are negatively regulated by NEP-2, we further screened for mutations that suppress the olfactory plasticity defect of *nep-2(pe379)*. One of these mutations, *pe1063*, strongly suppressed the defect of *nep-2* (Fig. 4A). Fine-mapping and rescue experiments showed that the causative gene was *C02F12.3*, which we named *snet-1* (suppressor of *nep-2*) (Fig. 4B and figs. S2, S3, S6B, and S8). *snet-1* is predicted to encode a small protein of 101 amino acids, with a signal sequence and dibasic cleavage sites typical of neuropeptide precursors (Fig. 4C). The predicted peptide region had partial similarity with *Aplysia* L11 peptide, which is a peptide secreted from the L11 neuron (fig. S9A) (*19*). Rescue experiments with truncated constructs suggested that the peptide region is important for the regulation of olfactory plasticity (fig. S9, B and C). When Venus was fused to the coding sequence, the fluorescence was observed in coelomocytes—the scavenger cells located in the body cavity—indicating that the protein is secreted by the action of the signal sequence (fig. S10, A, B, and G). Intracellular localization of SNET-1::Venus fusion protein and its dependence on UNC-104 kinesin-3 suggest that SNET-1 is transported to the neurites by kinesin-dependent anterograde transport and probably secreted there (fig. S10, C to F) (*20*). Olfactory plasticity defect of *nep-2* reappeared when *snet-1* was expressed by various cell-specific promoters in the *nep-2;snet-1* double mutant (Fig. 4D). These observations are consistent with SNET-1 being a secreted peptide and a substrate of the NEP-2 peptidase. Because null mutation of *snet-1* may not be fully epistatic to *nep-2* (Fig. 4A), there may be also other targets of NEP-2.

The *snet-1p::Venus* promoter fusion reporter was expressed in several neurons, including the sensory neurons ASK and the interneurons AIB, AIM, and PVQ (Fig. 3, G to I), suggesting that SNET-1 may be regulated by sensory inputs, such as sensation of the pheromone. Because overexpression of *snet-1* by increased gene dosage leads to defect in olfactory plasticity, quantitative regulation of the *snet-1* gene expression may be important (fig. S11). Using quantitative reverse transcription polymerase chain reaction (RT-PCR), we indeed found that the transcript of *snet-1* was increased in the adult animals of *daf-22* pheromone–less mutants as compared with the wild type (fig. S12). In the *daf-22* mutant background, *snet-1p::Venus* was expressed in ASI sensory neurons in addition to the cells observed in the wild-type background, and the supernumerary expression was suppressed by the addition of the pheromone to the culture plates (Figs. 3, J and K, and 4E). ASI neurons are known to regulate dauer larva formation, and expression of the genes *daf-7* transforming growth factor–β (TGFβ), *daf-28* insulin, and several chemoreceptors such as *srd-1*, *str-2*, and *str-3* in ASI is regulated by the dauer pheromone (*21*–*23*). Of particular interest is that expression of *str-3* and *daf-7* is regulated through the pheromone receptor SRBC-64/SRBC-66 on ASK sensory neurons in larvae (*24*), but mutants of these receptors did not show any defect in olfactory plasticity (fig. S13). These observations suggest that down-regulation of *snet-1* expression by the pheromone, probably in an SRBC-64/66–independent manner, results in promotion of olfactory plasticity by reducing the SNET-1 peptide signaling. In support of the notion that SNET-1 conveys the pheromonal signal, null mutation of *snet-1* suppressed the olfactory plasticity–defective phenotype of *daf-22* to the wild-type level (Fig. 4F).

Throughout the animal kingdom, greater population density leads to increased rates of dispersal behavior because high population density usually leads to competition for limited resources

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within a habitat (*5*–*7*). Population density–dependent olfactory plasticity may serve as one of such adaptive mechanisms. Our results indicate that *nep-2* regulates olfactory plasticity by acting in a cell-nonautonomous manner. Mammals possess seven neprilysin family proteins: neprilysin, *endothelin-converting enzyme 1* (ECE1), ECE2, phosphate-regulating neutral endopeptidase (PHEX), neprilysin-2, damage-induced neuronal endopeptidase (DINE), and Kell (*16*). Although little is known about the loss-of-function phenotypes of the neprilysin family proteins, recent biochemical studies revealed that neprilysin scavenges amyloid β peptide efficiently and with this mechanism can prevent Alzheimer's disease (*17*). On the basis of our observations, we propose a model in which SNET-1 peptide is secreted as an environmental signal that prevents olfactory plasticity and NEP-2 turns off the SNET-1 signals by degrading the excess peptides, creating a balance sensitive to environmental signals. By acting on an unknown receptor in the olfactory sensory circuit, SNET-1 negatively regulates olfactory plasticity (Fig. 4G and fig. S14). Given that *daf-22*, *nep-2*, and *snet-1* regulate the plasticity of the response to several types of odorants (fig. S3), the pheromonal information may also regulate other behaviors through the peptide signaling. Our identification of the SNET-1 pathway that regulates olfactory plasticity sheds light on the complexity of the regulatory network underlying simple sensory behavior in *C. elegans* and suggests similar regulation might be present in other animals.

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Materials and Methods
Figs. S1 to S14
References

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A Unifying Genetic Model for Facioscapulohumeral Muscular Dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) is a common form of muscular dystrophy in adults that is foremost characterized by progressive wasting of muscles in the upper body. FSHD is associated with contraction of D4Z4 macrosatellite repeats on chromosome 4q35, but this contraction is pathogenic only in certain "permissive" chromosomal backgrounds. Here, we show that FSHD patients carry specific single-nucleotide polymorphisms in the chromosomal region distal to the last D4Z4 repeat. This FSHD-predisposing configuration creates a canonical polyadenylation signal for transcripts derived from *DUX4*, a double homeobox gene of unknown function that straddles the last repeat unit and the adjacent sequence. Transfection studies revealed that *DUX4* transcripts are efficiently polyadenylated and are more stable when expressed from permissive chromosomes. These findings suggest that FSHD arises through a toxic gain of function attributable to the stabilized distal *DUX4* transcript.

Autosomal dominant FSHD (FSHD1; OMIM 158900) is a common form of muscular dystrophy, affecting 1 in 20,000 people, that is characterized by progressive and often asymmetric weakness and wasting of facial, shoulder girdle, and upper arm muscles (*1*). The disorder is most often caused by contraction of the D4Z4 macrosatellite repeat array in the subtelomeric region of chromosome 4q35 (*2*). This polymorphic macrosatellite repeat normally consists of 11 to 100 D4Z4 units, each 3.3 kb in size and ordered head-to-tail. Patients with FSHD1 have one repeat array of 1 to 10 units (Fig. 1A). At least one unit of D4Z4 is required to develop FSHD (*3*).

D4Z4 contraction needs to occur on a specific chromosomal background to cause FSHD. The chromosome 10q subtelomere contains an almost identical repeat array, but contractions on this chromosome are nonpathogenic (Fig. 1A). Translocated copies of the chromosome 4 and chromosome 10 repeat units are frequently encountered on either chromosome end (*4*). This complex genetic situation, in which genetically almost identical repeat units can be exchanged between both chromosomes with apparently discordant pathological consequences, has long hampered the identification of the disease mechanism.

Disease models were postulated in which D4Z4 repeat contractions cause chromatin remodeling and transcriptional deregulation of genes close to D4Z4. Indeed, contracted D4Z4 repeat arrays show partial loss of DNA methylation and of heterochromatic histone 3 lysine 9 trimethylation and heterochromatin protein 1γ markers consistent with a more open chromatin structure (*5*, *6*). Transcriptional up-regulation of genes proximal to D4Z4 was reported in FSHD1 patients (*7*) but could not be confirmed (*8*, *9*).

Exchanges between repeat units of chromosomes 4 and 10 occur much less frequently than anticipated: Most translocated repeat units are relicts of ancient translocation events between chromosomes 4q and 10q (*10*). Of the two distal chromosome 4q configurations, 4qA and 4qB, only contractions of the 4qA form led to FSHD1

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(*11*). Genetic follow-up studies unveiled consistent polymorphisms in the FSHD locus, resulting in the recognition of at least 17 genetic variants of distal 4q (*10*). Contractions in the common variant 4A161 cause FSHD1, whereas contractions in many other variants, such as the common 4B163, do not cause FSHD1 (Fig. 1A) (*12*). Thus, it appears that chromosome 4A161–specific sequence variants are causally related to FSHD.

Because at least one D4Z4 unit is necessary to cause disease, we reasoned that the minimal pathogenic region might reside in the first or the last unit. The distal unit of the D4Z4 repeat was recently shown to have a transcriptional profile that differs from internal units (*13*, *14*). Although the major transcript in each unit is the *DUX4* gene, which codes for a double homeobox protein, none of these transcripts seem to be stable,

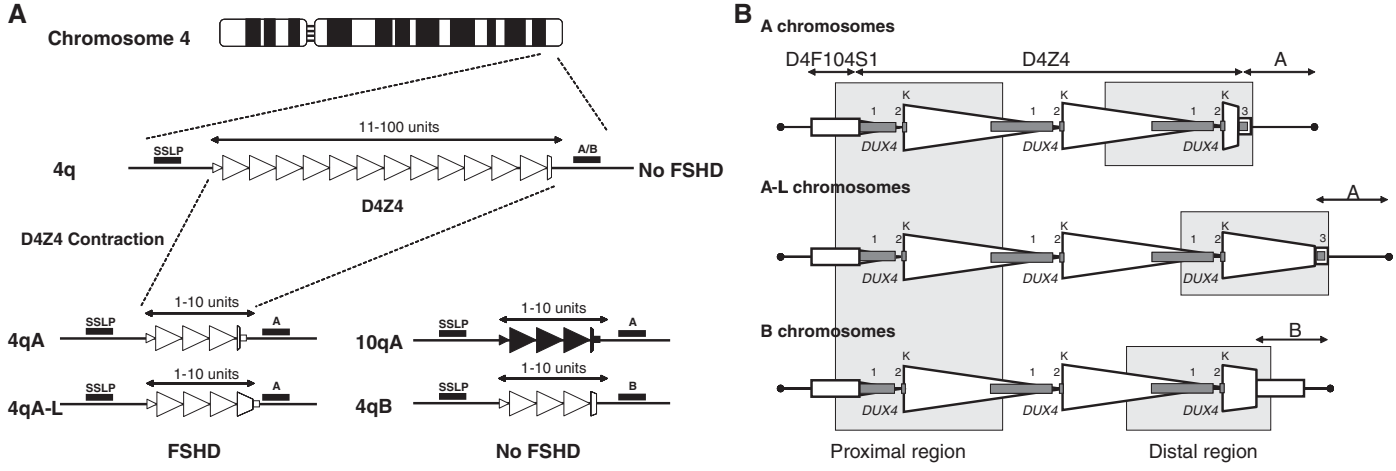


Fig. 1. Schematic overview showing the minimal genetic requirement for FSHD1. (**A**) The D4Z4 repeat array on chromosome 4q35 (open triangles) and its homolog on chromosome 10q26 (closed triangles), indicating the location of the simple sequence length polymorphism (SSLP) and 4qA/4qB polymorphisms that define the genetic background of the repeat. Patients with FSHD1 have a D4Z4 repeat array size of 1 to 10 units on 4qA but not on 4qB or 10q chromosomes. (**B**) Schematic of the D4Z4 repeat and flanking sequences on A, A-L (with an extended distal D4Z4 repeat unit), and B chromosomes. Each D4Z4 unit is defined by the *Kpn*I restriction site (K). The proximal and distal regions that were sequenced are indicated. The exons of *DUX4* are indicated as gray boxes numbered 1 to 3.

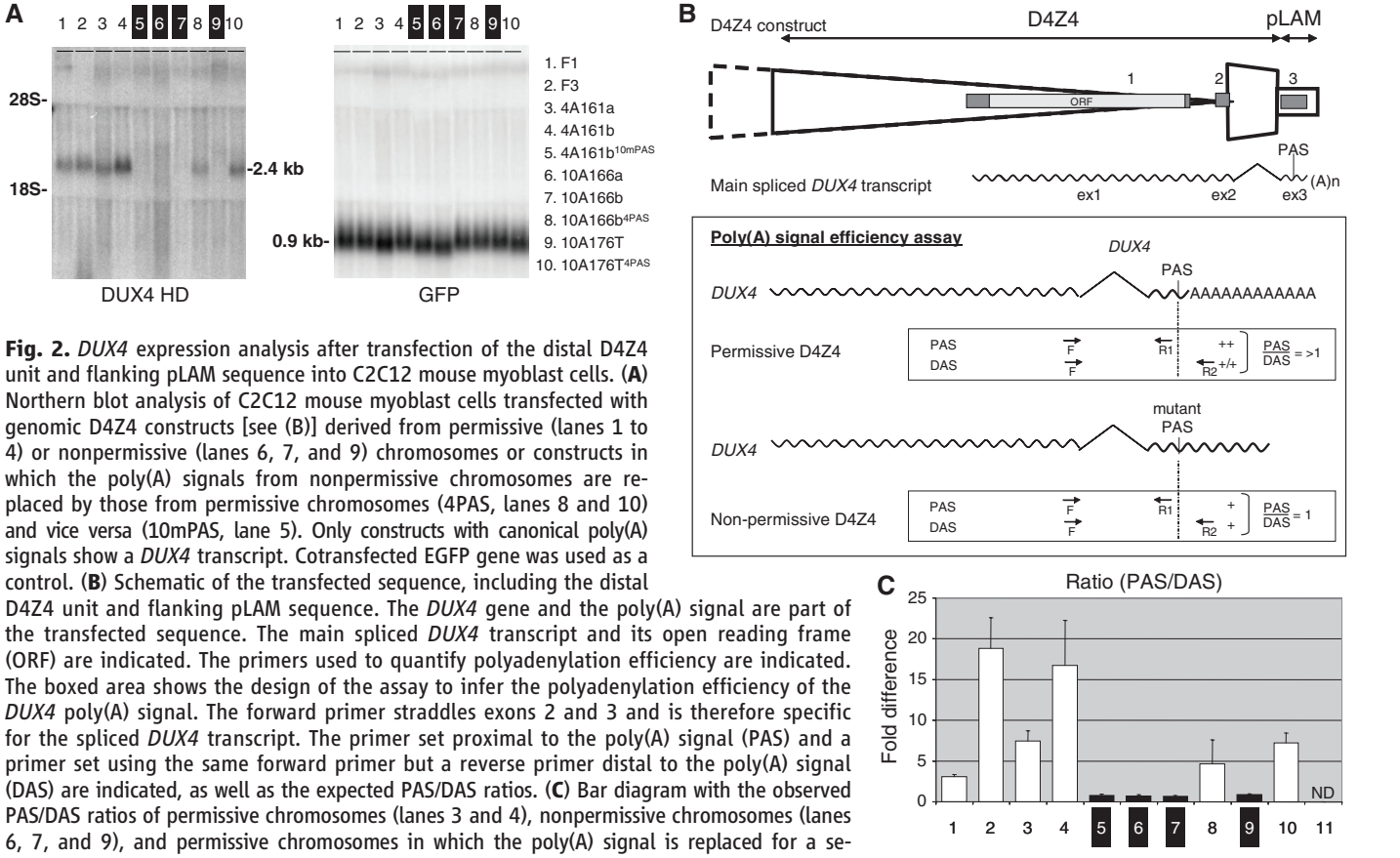


Fig. 2. *DUX4* expression analysis after transfection of the distal D4Z4 unit and flanking pLAM sequence into C2C12 mouse myoblast cells. (**A**) Northern blot analysis of C2C12 mouse myoblast cells transfected with genomic D4Z4 constructs [see (B)] derived from permissive (lanes 1 to 4) or nonpermissive (lanes 6, 7, and 9) chromosomes or constructs in which the poly(A) signals from nonpermissive chromosomes are replaced by those from permissive chromosomes (4PAS, lanes 8 and 10) and vice versa (10mPAS, lane 5). Only constructs with canonical poly(A) signals show a *DUX4* transcript. Cotransfected EGFP gene was used as a control. (**B**) Schematic of the transfected sequence, including the distal D4Z4 unit and flanking pLAM sequence. The *DUX4* gene and the poly(A) signal are part of the transfected sequence. The main spliced *DUX4* transcript and its open reading frame (ORF) are indicated. The primers used to quantify polyadenylation efficiency are indicated. The boxed area shows the design of the assay to infer the polyadenylation efficiency of the *DUX4* poly(A) signal. The forward primer straddles exons 2 and 3 and is therefore specific for the spliced *DUX4* transcript. The primer set proximal to the poly(A) signal (PAS) and a primer set using the same forward primer but a reverse primer distal to the poly(A) signal (DAS) are indicated, as well as the expected PAS/DAS ratios. (**C**) Bar diagram with the observed PAS/DAS ratios of permissive chromosomes (lanes 3 and 4), nonpermissive chromosomes (lanes 6, 7, and 9), and permissive chromosomes in which the poly(A) signal is replaced for a sequence derived from a nonpermissive chromosome (10mPAS, lane 5) or vice versa (4PAS, lanes 8 and 10), or of pathogenic chromosomes derived from families F1 and F3 (lanes 1 and 2). Lane 11 is a negative control. ND, not detected. Error bars represent values of quadruple experiments with standard errors of the mean.

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probably due to the absence of a polyadenylation signal in internal D4Z4 units. Spliced and unspliced transcripts of the *DUX4* gene in the last unit, however, use a unique 3′ untranslated region (UTR) in the pLAM region (*15*), which is immediately distal to this last unit (Figs. 1B and 2B) and which contains a poly(A) signal that presumably stabilizes this distal transcript (*13*, *14*). The *DUX4* transcript of the distal D4Z4 unit encompasses two facultative introns in the 3′UTR. When expressed in C2C12 muscle cells, DUX4 causes a phenotype compatible with molecular observations in FSHD (*16*). This distal *DUX4* transcript can be observed in FSHD1 myotubes but not in control myotubes (fig. S1) (*17*).

To investigate why the 4A161 chromosome is permissive for disease, we compared the sequence of the 4A161 chromosome with that of common, nonpermissive 4B163 and 10A166 chromosomes. We could not identify a sequence signature in the proximal D4Z4 unit of the repeat array that explained the permissiveness of the 4A161 chromosome (fig. S2). However, immediately distal to D4Z4, in the adjacent pLAM sequence, we found a polymorphism potentially affecting polyadenylation of the distal *DUX4* transcript. The *DUX4* poly(A) signal ATTAAA, which is commonly used in humans (*18*), is present on the permissive 4A161 chromosome, whereas the corresponding ATCAAA sequence on chromosome 10q is not known to be a poly(A) signal (fig. S2). Nonpermissive 4qB chromosomes, like 4B163, lack pLAM altogether, including this poly(A) site (Fig. 1B). Another nonpermissive 10qA chromosome (10A176T) (*10*) carries ATTTAA at this position, which is also not known as a poly(A) signal (figs. S2 and S3). In silico poly(A) signal prediction programs (*19*, *20*) also recognized the *DUX4* poly(A) signal in 4A161 but failed to identify potential poly(A) signals in nonpermissive chromosomes 10A166 and 10A176T.

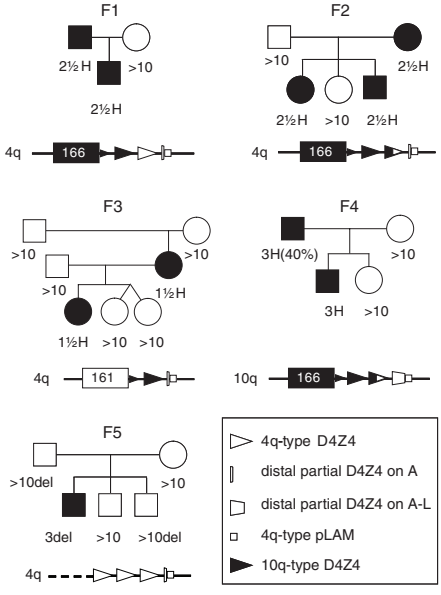


Fig. 3. Pedigrees of FSHD1 families with complex pathogenic chromosomes. Families F1 to F4 all carry a hybrid D4Z4 repeat (H) that commences with chromosome 10-type repeat units (closed triangles) but end with 4-type repeat units (open triangles). In family F3, a meiotic rearrangement between chromosomes 4 and 10 generated a short hybrid repeat structure on 4A161. In family F4, this pathogenic repeat is located on chromosome 10 and originates from a mitotic D4Z4 contraction in the mildly affected father that is transmitted to his affected son. Family F5 represents a disease chromosome in which, in addition to partial deletion of the D4Z4 repeat, the region proximal to the D4Z4 repeat is also deleted (del).

To explore whether these polymorphisms affect the distal *DUX4* transcript, we transfected the last D4Z4 unit and flanking pLAM sequence of permissive and nonpermissive chromosomes in C2C12 cells and assessed the stability of the distal *DUX4* transcript by Northern blot analysis (Fig. 2A). We also examined the relative potency of the poly(A) signals on the permissive and nonpermissive chromosomes in directing polyadenylation of the distal *DUX4* transcript. We studied polyadenylation site usage indirectly by using a quantitative reverse transcription polymerase chain reaction (*21*) in which we compared *DUX4* transcript levels proximal and distal of the poly(A) site (Fig. 2B). The use of the predicted poly(A) signal was verified by 3′ rapid amplification of cDNA ends (fig. S4). We also transfected constructs in which the poly(A) signal of permissive chromosomes was replaced by those of nonpermissive chromosomes, and vice versa. We found that *DUX4* transcripts were stable (Fig. 2A) and efficiently polyadenylated (Fig. 2C) when we used constructs from permissive chromosomes or when the poly(A) signal of a permissive chromosome was introduced on constructs derived from nonpermissive chromosomes. Consistently, when constructs derived from nonpermissive chromosomes were transfected, no *DUX4* transcripts could be detected on Northern blot and polyadenylation was inefficient. *DUX4* stability and polyadenylation efficiency decreased when the poly(A) signal of permissive constructs was replaced by nonpermissive sequences. Altogether, constructs with a bona fide poly(A) signal produced stable transcripts and showed polyadenylation efficiency that was 4 to 16 times as high as constructs with a mutation in the poly(A) signal. This suggests that increased polyadenylation, and hence stability, of the distal *DUX4* transcript may be centrally involved in FSHD pathogenesis.

We next studied FSHD1 patients with unusual hybrid D4Z4 repeat array structures that contain mixtures of 4-type and 10-type units. We identified four families (F1 to F4) with one or more individuals with FSHD1, carrying a contracted D4Z4 repeat array that commences with 10-type units and ends with 4-type units (Fig. 3). In family F3, we identified a patient with a de novo meiotic rearrangement between chromosomes 4q and 10q, leaving one and a half 10-type repeat units on a permissive 4A161 chromosome. In family F4, the mildly affected father is a mosaic FSHD1 patient (*22*) due to a mitotic contraction of such hybrid repeat array. The mosaic pathogenic repeat starts with two and a half 10-type D4Z4 units and ends with one and a half 4-type repeat units. This repeat array in the father was transmitted to his affected son, demonstrating its pathogenicity, and, unexpectedly, it was found to reside on chromosome 10 (fig. S6). Only the distal end of the D4Z4 repeat array was transferred to chromosome 10q, so that none of the FSHD candidate genes located proximal to the D4Z4 repeat array were cotransferred to chromosome 10 (fig. S6). This report of a FSHD1 family linked to chromosome 10 apparently precludes a key role for proximal 4q genes in the pathogenesis of FSHD. Altogether, all unusual FSHD1-causing repeat arrays reported here thus share the commonality of a terminal 4qA repeat unit with a directly adjacent pLAM sequence.

We also analyzed other disease-permissive chromosome 4 variants (fig. S7): 4A161L was previously described (*10*, *15*), whereas 4A159 and 4A168 are newly discovered uncommon permissive variants from a survey of >300 independent patients with FSHD. In addition, we studied >2000 control individuals and identified additional nonpermissive chromosome variants: 4B168, 10A164, and 10B161T (fig. S3). Thus, D4Z4 contractions on 4A161, 4A161L, 4A159, and 4A168 chromosomes are pathogenic, and D4Z4 contractions on 4B163, 4B168, 10A166, 10A164, 10B161T, and 10A176T chromosomes are nonpathogenic.

We sequenced the first and last D4Z4 units and flanking sequences in these newly identified permissive and nonpermissive chromosomes. (Fig. 1B and fig. S2). In support of our earlier data, there is no common sequence in the proximal D4Z4 region that unifies FSHD-permissive chromosomes. At the distal end, all permissive chromosomes differed very little in sequence and all contained a canonical *DUX4* poly(A) signal, whereas nonpermissive chromosomes showed much more sequence variation relative to the permissive chromosomes. The only exception, 4B163, has a D4Z4 sequence highly identical to 4A161 but, importantly, lacks the pLAM sequence (Fig. 1). The permissive 4A161L chromosome is identical to 4A161 but carries an extended D4Z4 sequence, preceding an identical pLAM sequence (Fig. 1B and fig. S2). Sequence analysis of the distal D4Z4-pLAM region of the pathogenic chromosome in our four families with complex repeat array structures showed a sequence identical to the permissive 4A161 sequence. Transfection experiments with D4Z4-pLAM sequences derived from the disease chromosomes of families F1 and F3 showed transcript stabilities and polyadenylation efficiencies of the distal *DUX4* transcript comparable to 4A161 chromosomes (Fig. 2B). This demonstrates that *DUX4* can also be efficiently produced from these chromosomes. Altogether, our study demonstrates that all patients with FSHD1 that came to our attention have an

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identical sequence in the last D4Z4 unit and immediately flanking pLAM sequence, and it shows that specific sequence variants unique to the permissive haplotypes confer pathogenicity to the repeat irrespective of its chromosomal localization (fig. S8).

Finally, this distal pLAM region is also preserved in individuals with FSHD1 in whom the deleted region extends proximally to the D4Z4 repeat array (F5 in Fig. 3), as well as in FSHD2 patients, who have a classical FSHD phenotype but show a similar local chromatin relaxation on a 4A161 chromosome independent of D4Z4 repeat array contraction (*6*, *23*).

Our study puts forward a plausible genetic model for FSHD. In this model, two polymorphisms create a polyadenylation site for the distal *DUX4* transcript, located in the pLAM sequence. In combination with the chromatin relaxation of the repeat, this leads to increased *DUX4* transcript levels. FSHD may arise through a toxic gain of function attributable to the stabilized distal DUX4 transcript. Our study thus not only explains the striking chromosome specificity of the disorder but also provides a genetic mechanism that may unify the genetic observations in patients with FSHD.

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The Genetic and Molecular Basis for Sunscreen Biosynthesis in Cyanobacteria

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Ultraviolet UV-A and UV-B radiation is harmful to living systems, causing damage to biological macromolecules. An important strategy for dealing with UV exposure is the biosynthesis of small-molecule sunscreens. Among such metabolites, the mycosporine and mycosporine-like amino acids (MAAs) are remarkable for their wide phylogenetic distribution and their unique chemical structures. Here, we report the identification of a MAA biosynthetic gene cluster in a cyanobacterium and the discovery of analogous pathways in other sequenced organisms. We have expressed the cluster in a heterologous bacterial host and characterized all four biosynthetic enzymes in vitro. In addition to clarifying the origin of the MAAs, these efforts have revealed two unprecedented enzymatic strategies for imine formation.

Mycosporines and mycosporine-like amino acids (MAAs) are ultraviolet (UV)–absorbing small molecules (λ_{max} = 310 to 360 nm) (*1*) that are synthesized by cyanobacteria, fungi, and eukaryotic micro- and macroalgae (*2*–*5*). These metabolites are also ingested and accumulated by numerous marine animals. Discovered and named for mediating light-induced fungal sporulation (*6*–*8*), mycosporines and MAAs are involved in many other biological processes, including UV photoprotection of organisms (*2*–*5*, *9*) and their embryos (*10*), osmotic regulation (*11*), and defense against oxidative stress (*12*). Additionally, their ability to prevent UV-induced skin damage in vivo (*13*) has led to their commercialization as Helioguard 365, a formulation containing MAAs shinorine and porphyra-334 that is used in skin care and cosmetic products.

Structurally this large natural-product family is divided into two groups: the mycosporines, which contain a single proteogenic or modified amino acid residue linked to a cyclohexenone core, and the MAAs, which have two such substituents (Fig. 1A). The amino acids are attached to the central ring through imine linkages, an arrangement best represented as a combination of resonance tautomers (Fig. 1B). This extensive conjugation facilitates absorption of UV (Fig. 1C). Fungi produce only mycosporines, whereas cyanobacteria, algae, and marine organisms can synthesize both mycosporines and MAAs.

The occurrence, distribution, and biology of mycosporines and MAAs have been subjects of intense investigation, but elucidation of the precise biochemical mechanisms underlying their functions has been hampered by a lack of knowledge regarding the molecular and genetic basis for their biosynthesis. Previous feeding studies with cyanobacteria and fungi revealed that the amino acid substituents were added in the later stages of biosynthesis and suggested, but did not prove, that the mycosporine core might originate from shikimate pathway intermediate 3-dehydroquinate (*14*, *15*). Despite these efforts, no genes have been definitively linked to mycosporine or MAA production in any organism.

We attempted to locate candidate biosynthetic gene clusters using a genome mining approach prompted by the discovery of genes encoding shikimate pathway enzymes in the genome of sea anemone *Nematostella vectensis* (*16*), including a dehydroquinate synthase homolog (DHQS) adjacent to an *O*-methyltransferase (O-MT). Recognizing that a methyltransferase was likely involved in mycosporine assembly, we considered the DHQS and O-MT to be candidate biosynthetic genes. BLAST searches with both protein sequences revealed clustered homologs in genomes of dinoflagellates, cyanobacteria, and fungi (*17*).

Examining the hits in their genomic contexts revealed that open reading frames (ORFs) encoding DHQSs and O-MTs were accompanied by a third conserved ORF, usually annotated as a hypothetical protein. A conserved domain search using this gene revealed homology to the adenosine triphosphate (ATP)–grasp superfamily. As these enzymes catalyze peptide bond formation, we reasoned that this gene product might be involved in amino acid installation. We also noted genetic variations between cyanobacterial and fungal clusters consistent with the structural differences between cyanobacterial MAAs and fungal mycosporines; cyanobacteria had a second

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putative amide bond–forming enzyme, either an ATP-grasp or nonribosomal peptide synthetase (NRPS) homolog, whereas fungi contained additional enzymes or domains annotated as reductases. Recently, others identified the same DHQS and O-MT genes, but did not note the conserved ATP-grasp homolog nor demonstrate a genetic or biochemical connection to MAA production (*18*).

The cyanobacterium *Anabaena variabilis* American Type Culture Collection (ATCC) 29413 is an established producer of the MAA shinorine (Fig. 1A) (*19*) and contains a putative gene cluster consisting of four ORFs (Fig. 2A). We hypothesized that DHQS homolog Ava_3858 and O-MT Ava_3857 would assemble 4-deoxygadusol, a cyclohexenone natural product that resembles the mycosporine core (*20*), and that ATP-grasp homolog Ava_3856 and NRPS-like enzyme Ava_3855 might be responsible for attaching glycine and serine to 4-deoxygadusol via imine linkages (Fig. 2B). We demonstrated a direct connection of these genes to MAA biosynthesis by cloning the entire 6.5-kb shinorine biosynthetic gene cluster, as well as two truncated constructs lacking either Ava_3855 or both Ava_3855 and Ava_3856, into the heterologous host *Escherichia coli*. After inducing gene expression, we detected shinorine production only in the strain harboring the complete gene cluster (Fig. 2C). Strains containing truncated clusters produced mycosporine glycine (Ava_3855 truncation) and 4-deoxygadusol (Ava_3855/3856 truncation). All three products were isolated from culture extracts and characterized (*17*). These experiments clearly demonstrate the involvement of this gene cluster in shinorine production and confirm the identities of key biosynthetic intermediates. The synthesis of shinorine by *E. coli* suggests that no additional genes are required beyond those encoded in the *A. variabilis* gene cluster and that the pathway originates from a metabolic intermediate common to both organisms. Finally, these results provide strong evidence that homologous clusters are involved in mycosporine and MAA synthesis.

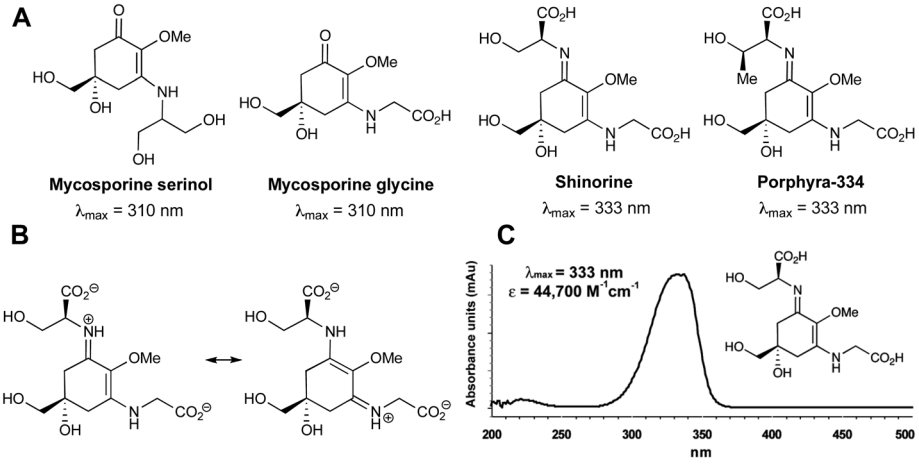


Fig. 1. Mycosporines and MAAs. (**A**) Chemical structures of representative mycosporines and MAAs from fungi (mycosporine serinol) and cyanobacteria (mycosporine glycine, shinorine, and porphyra-334). (**B**) Shinorine resonance tautomers. (**C**) UV-visible absorbance spectrum of shinorine.

Seeking an understanding of the chemistry underlying MAA assembly, we characterized each of the shinorine biosynthetic enzymes in vitro. Because of past successes expressing enzymes from the shinorine-producing cyanobacterium *Nostoc punctiforme* ATCC 29133 (*18*) in *E. coli*, we chose to examine the DHQS homolog (NpR5600) and O-MT (NpR5599) from this organism. Unexpectedly, we failed to observe any 4-deoxygadusol production when the putative substrate 3-dehydroquinate was incubated with both enzymes and typical cofactors *S*-adenosylmethionine (SAM), nicotinamide adenine dinucleotide (NAD^+), and Co^{2+} (*21*, *22*). Closer examination of the homology between NpR5600 and canonical DHQSs revealed that the active-site residues of enzymes associated with mycosporine biosynthesis resemble DHQS homolog 2-*epi*-5-*epi*-valiolone synthase (EVS) rather than a canonical DHQS (*18*, *22*, *23*), which suggests that these enzymes might share a common substrate, pentose phosphate pathway intermediate sedoheptulose 7-phosphate (SH 7-P). Gratifyingly, treatment of SH 7-P with NpR5600 and NpR5599 in the presence of SAM, NAD^+, and Co^{2+} produced a single product, 4-deoxygadusol. Kinetic analysis and a mechanistic proposal are detailed in the supporting online material (SOM; figs. S5 to S7 and scheme S1). This result is consistent with previous feeding studies, yet contradicts a longstanding assumption that MAA biosynthesis involves a shikimate pathway intermediate.

We next investigated the final steps of shinorine biosynthesis, installation of glycine and serine substituents. Imines, or Schiff bases, are important functional groups in biological systems and are typically formed by condensation of an amine nucleophile and a carbonyl electrophile in an ATP-independent manner. This type of imine formation is utilized by many enzyme families, including class I aldolases and pyridoxal phosphate (PLP)–dependent enzymes. Previous studies suggested that a distinct mechanism might operate in mycosporine biosynthesis, as mycosporine

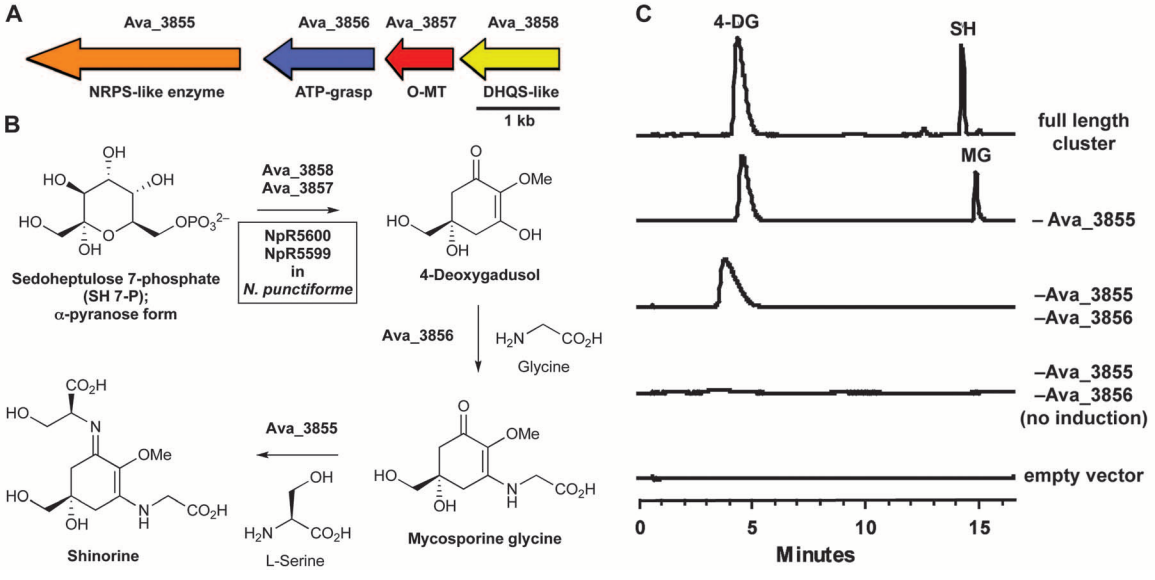


Fig. 2. Biosynthesis of shinorine. (**A**) The putative shinorine gene cluster from *A. variabilis*. (**B**) Biosynthetic pathway for the assembly of shinorine from sedoheptulose-7-phosphate. (**C**) HPLC traces of methanol extracts from *E. coli* induced with 500 μM IPTG at 15°C (310 nm). SH, shinorine; MG, mycosporine glycine; 4-DG, 4-deoxygadusol.

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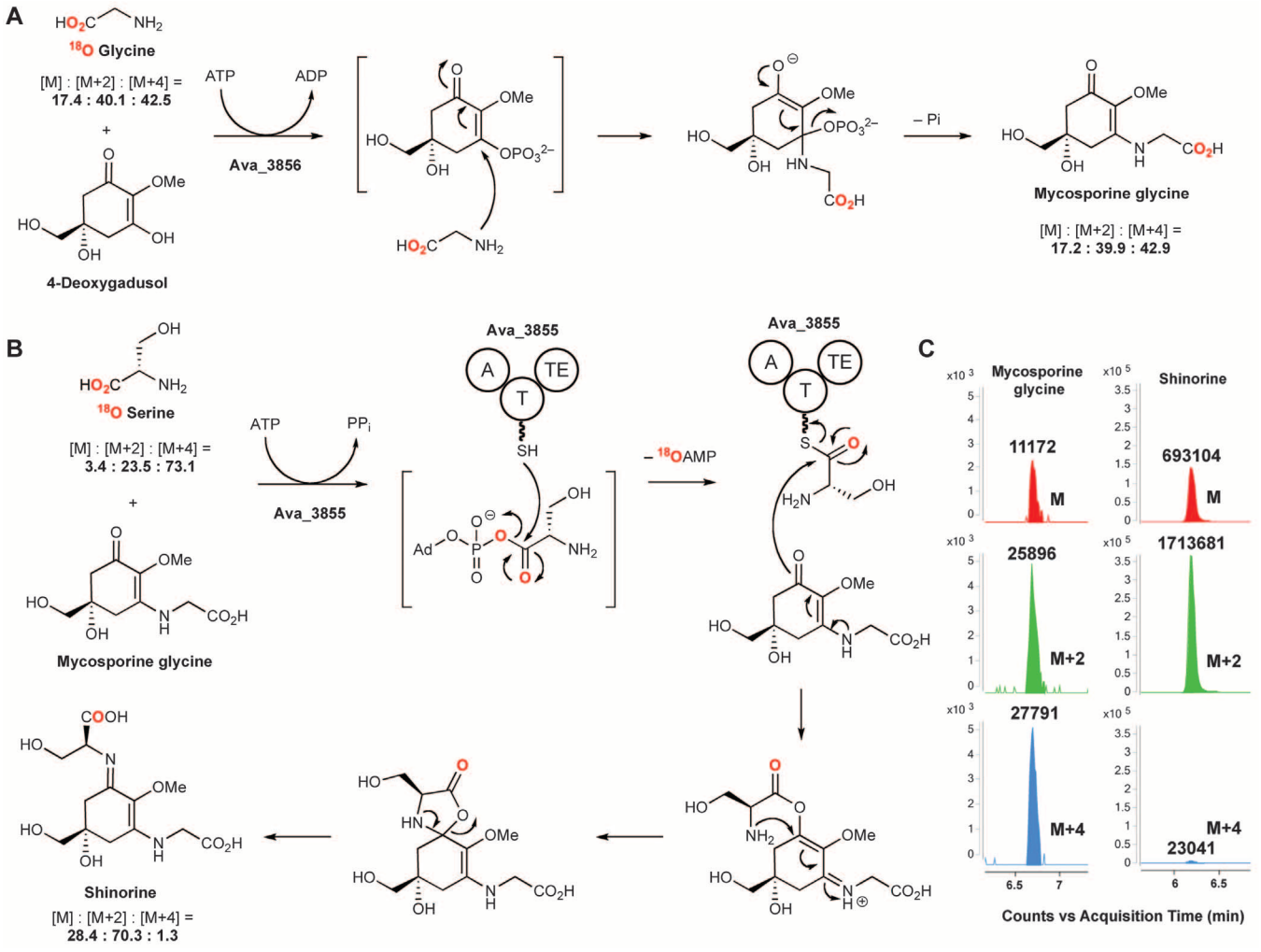


Fig. 3. ^{18}O-labeling experiments. (**A**) Reaction of 4-deoxygadusol and [^{18}O] glycine with ATP-grasp homolog Ava_3856. (**B**) Reaction of mycosporine glycine and [^{18}O] serine with NRPS-like enzyme Ava_3855. (**C**) Extracted ion chromatograms from LC-MS analysis of Ava_3856 and Ava_3855 assays.

glycine will not condense with amino acids to form MAAs (*24*). Accordingly, the above heterologous expressions confirmed that homologs of ATP-dependent peptide bond–forming enzymes were required for amino acid attachment. As the imine linkages in mycosporines and MAAs differ from peptide bonds, the mechanisms underlying these ligation steps were of great interest.

Attempts to overexpress putative imine-forming genes from the *N. punctiforme* shinorine cluster were unsuccessful; we therefore returned to *A. variabilis*, cloning both ATP-grasp family member Ava_3856 and NRPS-like enzyme Ava_3855 and overexpressing the corresponding proteins in *E. coli*. High-performance liquid chromatography (HPLC) assays of Ava_3856 confirmed its ability to convert 4-deoxygadusol and glycine into mycosporine glycine in the presence of ATP and Mg^{2+} cofactors. Two potential mechanisms could account for this transformation, one involving phosphorylation of 4-deoxygadusol followed by 1,4-addition of the amino acid nitrogen to the activated cyclohexenone, and the other invoking phosphorylation of glycine to give an acyl phosphate that could be attacked by 4-deoxygadusol. The acylated enol intermediate formed could then undergo an O-to-N rearrangement driven by an intramolecular 1,4-addition of the glycine amino group to the cyclohexenone core (scheme S2).

To distinguish between these possibilities, we performed an assay with ^{18}O-labeled glycine and analyzed the isotope content of mycosporine glycine using high-resolution liquid chromatography–mass spectrometry (LC-MS). The distribution of ^{18}O in the product was identical to that of the glycine starting material (Fig. 3A), which indicated that Ava_3856 phosphorylates 4-deoxygadusol rather than glycine, as the latter would be accompanied by loss of one ^{18}O atom via elimination of phosphate. Phosphorylation of 4-deoxygadusol was unexpected; although members of the ATP-grasp family use various nucleophilic partners, including amines and thiols, the electrophiles activated are typically carboxylic acids (*25*).

The final enzyme characterized was Ava_3855, an NRPS-like enzyme containing adenylation (A), thiolation (T), and thioesterase (TE) domains (*26*). HPLC assays confirmed its role in converting mycosporine glycine and serine to shinorine; activity was dependent on the presence of ATP, and the alternate amino acid threonine was not utilized by the enzyme. As with Ava_3856, a key mechanistic question regarding the action of Ava_3855 was the identity of the electrophilic reaction partner. In this case, activation of serine as an acyl adenylate was predicted on the basis of conserved A domain active-site residues (*27*) and confirmed by measuring the exchange between ATP and ^{32}P-labeled inorganic phosphate. Covalent attachment of radiolabeled serine onto the T domain of Ava_3855 was also demonstrated.

To establish that the serine thioester was on pathway to shinorine, we assayed Ava_3855 using [carboxy-$^{18}O_2$]serine. In this case, the product LC-MS revealed a shift of two mass units, indicating that Ava_3855 indeed operates through activation of the serine carboxylate; adenylation and loading by the A and T domains is accompanied by loss of ^{18}O as adenosine monophosphate (AMP). The mechanism of imine formation may involve TE domain–catalyzed formation of an enol ester intermediate, which could undergo

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subsequent O-to-N rearrangement via 1,4-addition of the serine nitrogen to the activated cyclohexenimine core (Fig. 3B). We favor this pathway, as it is consistent with the poor electrophilicity of mycosporine glycine (*24*); an alternative mechanism for Ava_3855 involving a direct condensation is detailed in scheme S3.

The in vitro characterization of Ava_3856 and Ava_3855 reveals two distinct, yet complementary, mechanisms of ATP-dependent enzymatic imine formation that differ from conventional chemical methods and biochemical mechanisms. These enzymes have evolved from peptide bond–forming catalysts in distinct ways: ATP-grasp homolog Ava_3856 generates a new type of electrophile using vinylogous acid activation, and NRPS-like enzyme Ava_3855 likely employs an unusual release mechanism. The recruitment of ATP-dependent peptide bond–forming enzymes in this manner is so far unprecedented in natural product biosynthesis and defines a new biosynthetic logic for imine construction. A short four-enzyme pathway thus converts a primary metabolite from the pentose phosphate pathway into a widely distributed class of small-molecule biological sunscreens using mechanistically elegant chemistry.

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Materials and Methods
Tables S1 to S5
Figs. S1 to S10
Schemes S1 to S3
References

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A General Mechanism for Network-Dosage Compensation in Gene Circuits

Murat Acar,[1]*† Bernardo F. Pando,[2]* Frances H. Arnold,[3] Michael B. Elowitz,[1,4] Alexander van Oudenaarden[2,5]

Coping with variations in network dosage is crucial for maintaining optimal function in gene networks. We explored how network structure facilitates network-level dosage compensation. By using the yeast galactose network as a model, we combinatorially deleted one of the two copies of its four regulatory genes and found that network activity was robust to the change in network dosage. A mathematical analysis revealed that a two-component genetic circuit with elements of opposite regulatory activity (activator and inhibitor) constitutes a minimal requirement for network-dosage invariance. Specific interaction topologies and a one-to-one interaction stoichiometry between the activating and inhibiting agents were additional essential elements facilitating dosage invariance. This mechanism of network-dosage invariance could represent a general design for gene network structure in cells.

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It is believed that in the transition between haploid and diploid forms of life cells use a volume-mediated compensation mechanism to keep the concentrations of transcription factors constant as cell volume increases with ploidy (*2*). However, this mechanism cannot subdue the effects of global expression variation and genome duplication or loss events because they affect cellular phenotypes independently of cell volume. For example, variability in ribosome numbers can cause substantial fluctuations in global expression levels. These observations raise the question of whether there are alternative layers of dosage compensation mechanisms independent of external factors such as cell volume. To what extent would network activity be robust to alterations in network dosage if we fixed cell volume and therefore excluded its compensatory effect? Could there be a molecular mechanism intrinsic to the network structure that helps cells diminish the effects of dosage variations? Despite the fundamental nature of these questions, what these mechanisms are and how they can be implemented has remained unclear.

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Gal2p. In its active form, Gal3p sequesters the Gal80p repressor to the cytoplasm, indirectly promoting transcription (*13*, *14*). Except for the constitutive *GAL4* promoter, the activities of the different GAL pathway promoters are similar to each other (*7*). To quantify the activity of the GAL pathway at the single-cell level, we used the yellow fluorescent protein (YFP) driven by the *GAL1* promoter as our reporter and measured expression profiles at different galactose concentrations by using flow cytometry (Fig. 1, A and B). We interpreted these experimental results in the context of an effective model (*15*).

We observed similar inducibility profiles between haploid and diploid strains that contain the same reporter system (Fig. 2A), demonstrating that the system is invariant to ploidy changes. To dissect how network-dosage variations affect the inducibility of the network in the absence of volume effects, we systematically reduced the number of copies of the four regulatory genes in the GAL network from two to one in diploid backgrounds by using *KanMX4* and *NatMX4* cassettes (*15*), obtaining 16 different diploid yeast strains, including the hemizygous and the wild-type strains that have all four genes at one and two copies, respectively (*15*).

Halving the dosage of *GAL3* dramatically reduced wild-type inducibility levels, whereas halving the dosage of *GAL80* made the cells need less galactose for full induction (Fig. 2B). Varying *GAL2* or *GAL4* dosage levels did not have a large effect on network activity (Fig. 2C).

To comprehensively explore the degree of dosage compensation in the GAL network, we measured the inducibility profiles of all 16 strains, grouped the measurements in four dosage-perturbation orders, and compared the profiles to one another (Fig. 3A) (*15*). We observed similar inducibility profiles for the fourth-order hemizygous strain and the wild-type strain, implying the presence of network-dosage invariance in the GAL network, even in the absence of volume-mediated compensation effects (Fig. 3, A and B).

To determine the relative importance of each regulatory gene in affecting the wild-type inducibility levels, we quantified the average contribution of the second copy of each gene to inducibility (*15*). Figure 3C depicts the greater importance of *GAL3* as an activator and *GAL80* as an inhibitor compared with the relatively smaller contributions of *GAL2* and *GAL4* to the inducibility profiles (*15*). These results suggest that it may be possible to build a dosage-invariant network

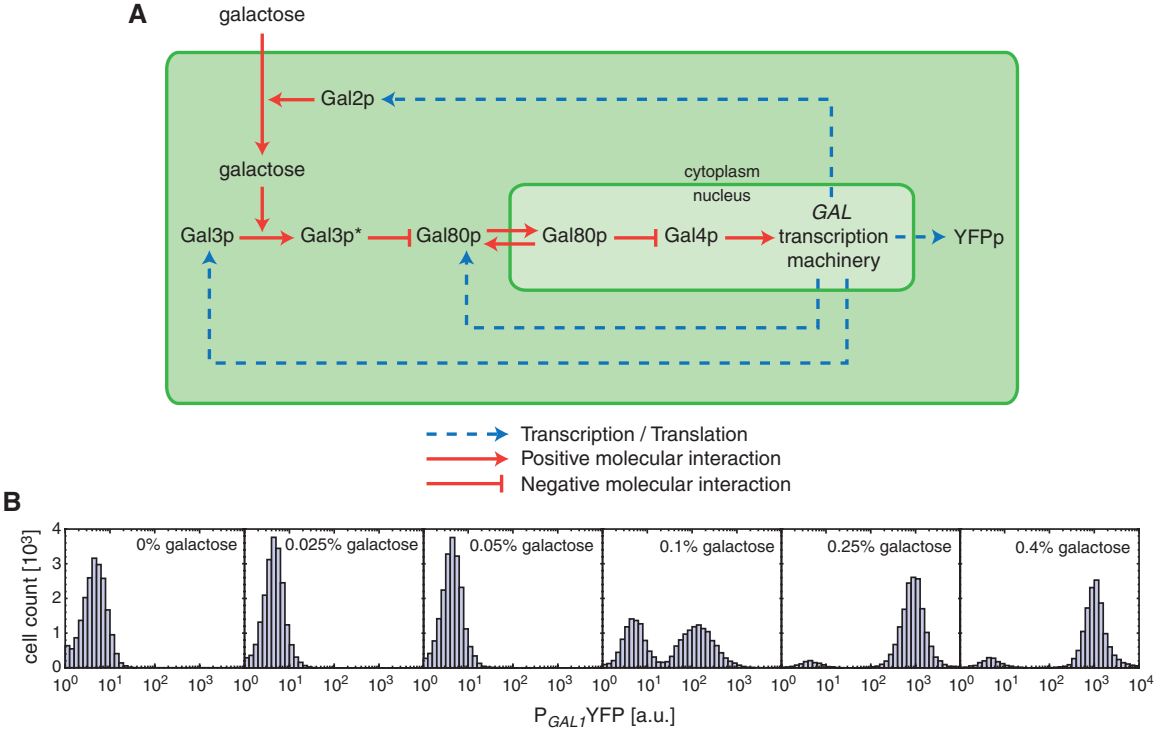


Fig. 1. The galactose utilization pathway as a model gene network and bistability as a quantitative phenotype. (**A**) Gal3p* represents the galactose-bound, active form of Gal3p. The shuttling of Gal80p between the cytoplasm and the nucleus is denoted by the bi-directional red arrows. The dotted blue arrows show how the transcriptional feedback loops are established through Gal2p, Gal3p, and Gal80p. (**B**) Histograms show induction profiles of the wild-type galactose pathway for different galactose concentrations. a.u., arbitrary units.

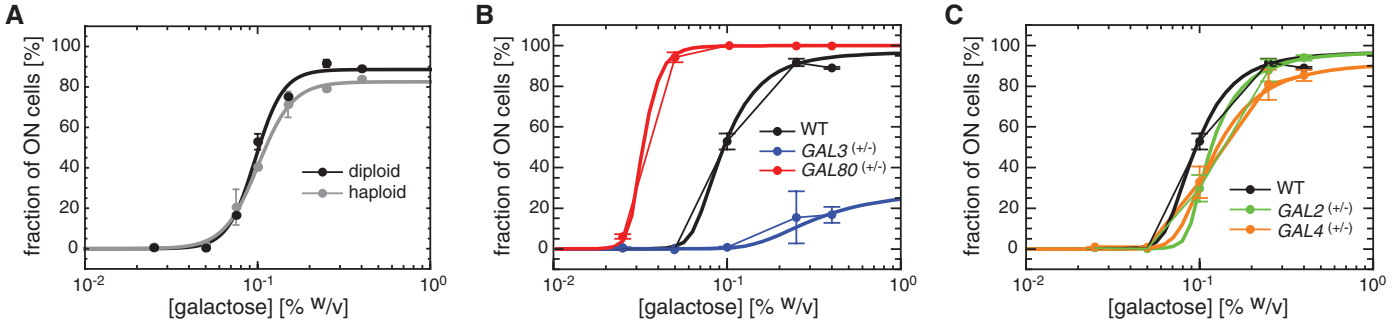


Fig. 2. Haploid-diploid comparison and measurement of the contribution of each regulatory gene to network inducibility. Error bars indicate SEM. (**A**) Fraction of on cells as a function of galactose concentration for both diploid and haploid strains. The solid lines are guides to the eye constructed by fitting a sigmoidal function to the data. (**B**) The inducibility profile of the GAL network heterozygous in *GAL3* (blue) or *GAL80* (red) relative to the wild-type (WT) profile (black). (**C**) The inducibility profile of the GAL network heterozygous in *GAL2* (green) or *GAL4* (orange) relative to the wild-type profile (black). In both (B) and (C), the thick solid lines represent the model best fit to the five different inducibility profiles.

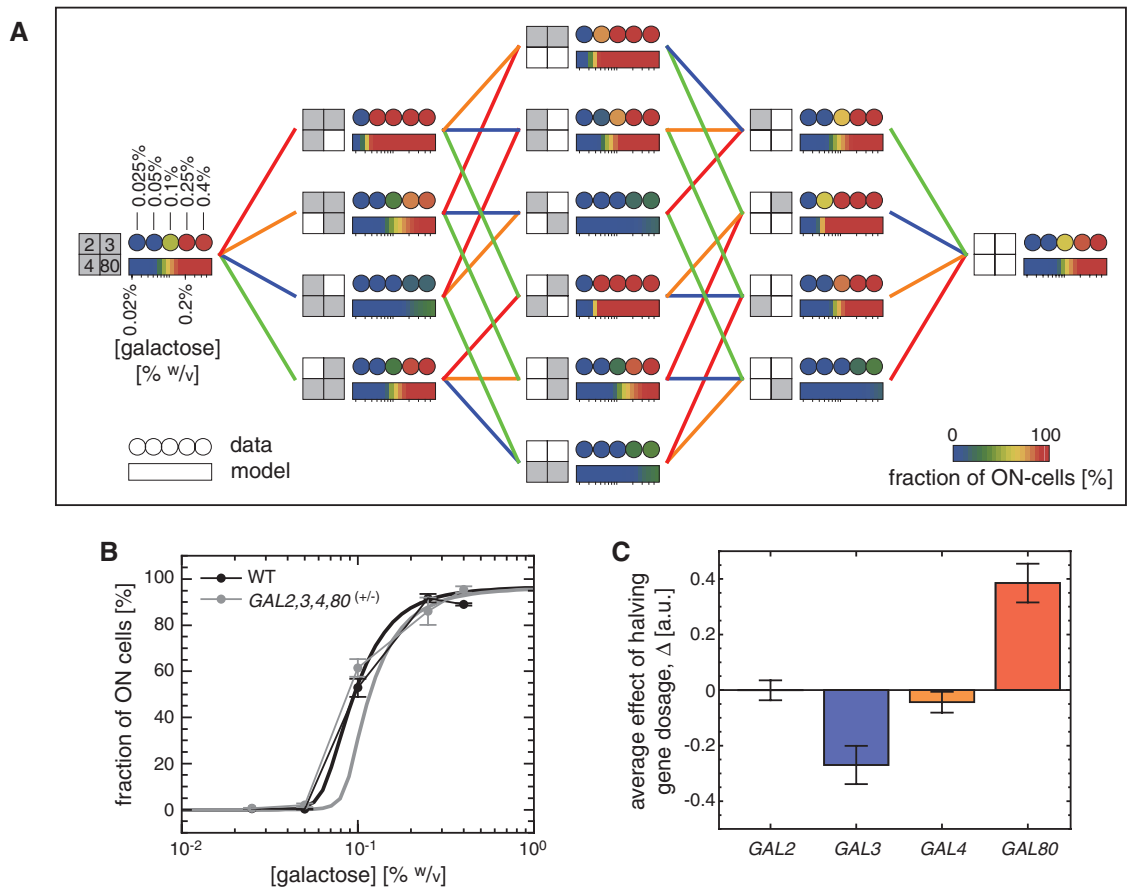


Fig. 3. Systematic dosage variations and network-dosage compensation. (**A**) The color of each circle represents the network inducibility level. The rectangular, color-coded bars reflect the predictions of the model based on the best fit to the data presented in Fig. 2, B and C. The genetic background of each strain is specified by a big square at its immediate left. The small squares represent the four regulatory genes of the *GAL* network. Gray color marks the presence of two copies of a specific gene, and white marks one copy of a specific gene. A line between two strains indicates that the two genetic backgrounds differ by a single copy of a specific gene, and the color of the line codifies that gene (blue for *GAL3*, red for *GAL80*, green for *GAL2*, and orange for *GAL4*). (**B**) The similarity between the inducibility profiles of the wild-type strain (black) and the strain containing one copy of each regulatory gene (gray). The thick solid lines represent the model predictions. (**C**) Average contribution of the second copy of each regulatory gene to network inducibility (*15*). Error bars indicate SEM.

by using only two components, but they do not by themselves indicate how the wiring topology of the network components contributes to network-dosage invariance.

To pinpoint the minimal general conditions that can facilitate dosage invariance in the absence of volume effects, we moved away from the specific case of the GAL pathway and analyzed generic network structures consisting of a set of genes all regulated by the same factor (*15*). We first found that any network with only one component cannot be dosage invariant. For networks with two components, dosage invariance is possible only if the components have opposite regulatory signs (i.e., if one is an activator and the other is an inhibitor).

To further explore how certain wiring topologies of the two-component generic networks would affect dosage invariance, we performed numerical investigations on the possible network topologies and analyzed their inducibility properties. Alternative network configurations are achieved on the basis of the following interaction topologies: the activator indirectly activates transcription, the activator directly activates transcription, the inhibitor gives up its direct-repressor role, and the activator assumes a direct-activator role (Fig. 4A). Each interaction topology is represented by a four-parameter functional form (Fig. 4A).

We randomly sampled the parameters characterizing these forms over large ranges and fed them into the quantitative model to obtain numerical inducibility curves corresponding to the networks carrying one or two copies of the network genes (*15*). For each pair of these numerical curves, we calculated the level of dosage invariance by quantifying the area between the two curves, large areas corresponding to large penalties to network-dosage invariance and vice versa (Fig. 4B). In principle, a high degree of dosage invariance can be observed at several different inducibility levels. For example, a biological network always staying in its off state is network-dosage invariant, but it lacks the ability to respond to signals of any kind. Thus, it is important to determine whether a dosage-compensated system is also inducible or not. We quantified the relative inducibility levels of our numerical curves relative to a reference induction profile. Large differences from the reference curve corresponded to large penalties to inducibility (Fig. 4B). An examination of the dot plots reveals that the topologies at left and right exhibit both dosage invariance and inducibility for a wide range of parameter sets. The specific interaction configuration in the two networks is essential for the systems to display such behavior (Fig. 4A). However, the choice between activator and inhibitor in directly influencing transcription is not essential, so long as the other component regulates indirectly.

The green areas in Fig. 4B enclose the parameter sets corresponding to dosage-invariant and inducible networks (low penalties in both axes). For each point populating these areas, we extracted out the values of the four parameters (Fig. 4, C and D) (*15*). The parameter quantifying the nonlinearity of the interaction between the inhibiting and activating agents (α in Fig. 4C and β in Fig. 4D) was the only one severely restricted in its values, which displayed a narrow distribution centered around one. Thus, the effective stoichiometry of the interaction between the activating and inhibiting agents has to be close to one-to-one for a system that is both inducible and network-dosage invariant (*15*).

To understand why an inducible, network-dosage invariant system requires these specific interaction topologies and a one-to-one stoichiometry, consider how the system would respond to coordinated changes in the activator and inhibitor levels. For the system in the center of Fig. 4A, the output depends on independent contributions from the activator and the inhibitor. For compensation, the increase in the activator concentration would have to be exactly compensated by the down-regulation effect by the

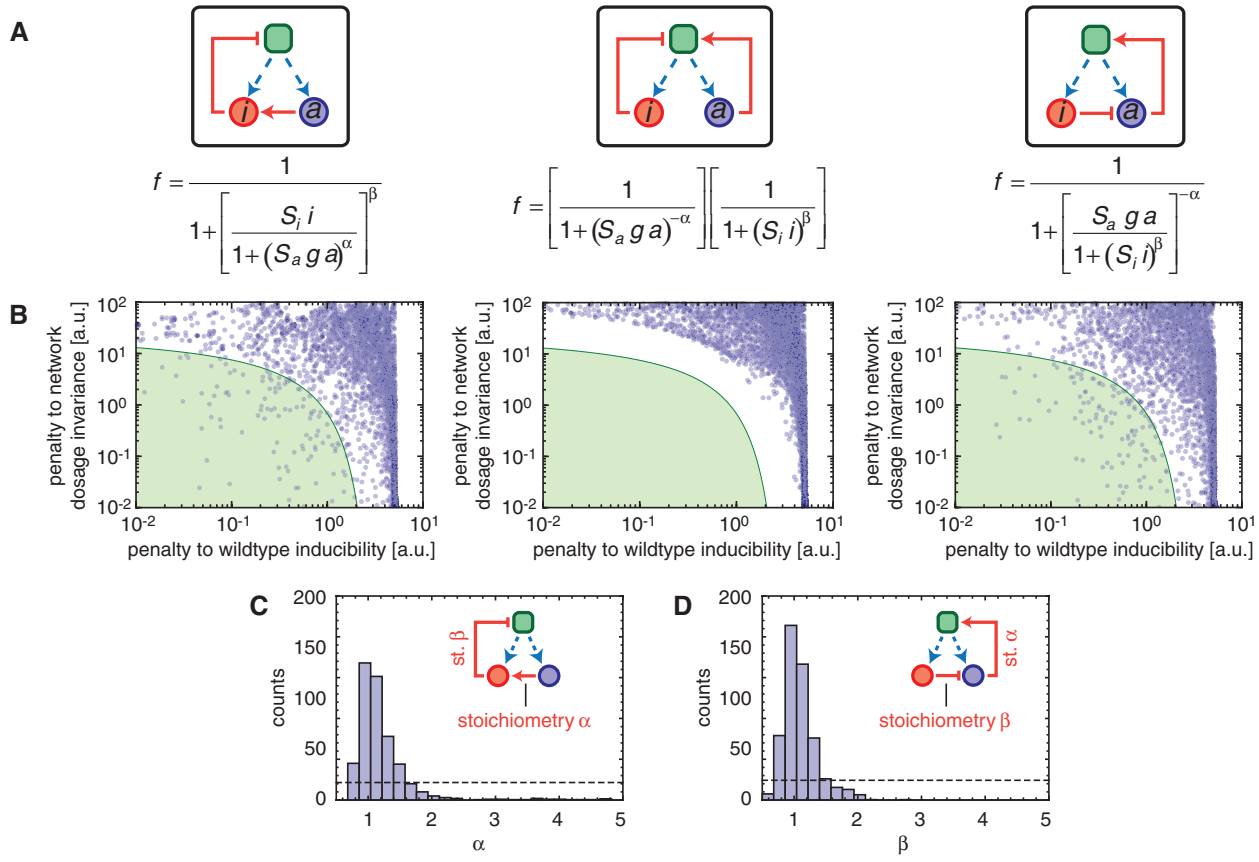


Fig. 4. Numerical analysis of general network features producing an inducible and network-dosage invariant system. (**A**) Each functional form represents the relationship between the fraction of transcriptionally active cells and the total concentrations of the activating (a) and inhibiting (*i*) agents. Blue and red circles represent activating and inhibiting agents, respectively. Dashed blue arrows denote the transcriptional production of the network components. The green square represents a transcriptional center. Pointing red arrows show direct activation, whereas blunt red arrows represent inhibition. Each configuration is described by four parameters: the scales of action of the activator and inhibitor (S_a and S_i, respectively) and coefficients (α and β) quantifying the typical nonlinearity of the interaction with downstream components. (**B**) For each configuration depicted in (A), the degree of inducibility and network-dosage invariance of systems are plotted on the *x* and *y* axes, respectively. The green region corresponds to systems that are both inducible and network-dosage invariant. (**C**) For the left configuration in (A), histogram of the parameter values corresponding to the green region shown in (B). (**D**) As in (C) but for the right configuration shown in (A). In (C) and (D), the dotted lines show what one would expect had the parameters had no effect in determining whether the system was in the green region or not.

inhibitor. However, given the nonlinear effect of each component on output, compensation cannot be maintained over a large range of input levels. The system thus fails to be both inducible and network-dosage invariant. For the other systems analyzed, when the one-to-one stoichiometry condition is satisfied, an increase in the activator concentration is compensated by an increase in the inhibitor, because the regulation function is dependent on just the ratio of these levels (*15*).

The network-dosage invariant GAL system satisfies the dosage compensation requirements identified by the minimal model: The interaction topology between its activator (*GAL3*) and inhibitor (*GAL80*) is similar to the topology depicted in Fig. 4, left. In addition, it has been experimentally shown (*16*) that *GAL3* and *GAL80* interact with one-to-one stoichiometry. These observations further validate our findings.

By using a constitutive promoter (*CYC1*) to eliminate the feedback regulation through the *GAL3* and *GAL80* genes, earlier work (*17*) measured the contribution of the *GAL3* and *GAL80* feedback loops to the noise in the network activity. It was found that without the feedback regulation the activity of the GAL network became noisier compared with activity of the wild-type network. Here, we have kept feedback regulation intact by maintaining at least one copy of the *GAL3* and *GAL80* genes and probed the effect of gene and network-dosage variations on the network activity, elucidating the contribution of network structure on dosage compensation.

These results provide a volume-independent mechanism that is sufficient for network-dosage invariance. The mechanism requires at least two network components: one positive and one negative regulator. These components have to interact with a one-to-one effective stoichiometry and have specific topologies allowing only one of them to directly affect transcription. This type of interaction topology is frequently observed (*18*–*21*) in natural gene circuits that use sequestration-based signal transduction schemes. Robust network properties such as network-dosage invariance might be selected over evolutionary time scales; therefore, network-dosage invariance could represent a general design principle for gene network architecture in cells (*22*–*29*).

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Supporting Online Material

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Materials and Methods
Figs. S1 to S8
Tables S1 to S5
References and Notes

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A *Vibrio* Effector Protein Is an Inositol Phosphatase and Disrupts Host Cell Membrane Integrity

Christopher A. Broberg, Lingling Zhang, Herman Gonzalez, Michelle A. Laskowski-Arce, Kim Orth*

The marine bacterium *Vibrio parahaemolyticus* causes gastroenteritis in humans and encodes the type III effector protein VPA0450, which contributes to host cell death caused by autophagy, cell rounding, and cell lysis. We found that VPA0450 is an inositol polyphosphate 5-phosphatase that hydrolyzed the D5 phosphate from the plasma membrane phospholipid phosphatidylinositol 4,5-bisphosphate. VPA0450 disrupted cytoskeletal binding sites on the inner surface of membranes of human cells and caused plasma membrane blebbing, which compromised membrane integrity and probably contributed to cell death by facilitating lysis. Thus, bacterial pathogens can disrupt adaptor protein–binding sites required for proper membrane and cytoskeleton dynamics by altering the homeostasis of membrane-bound inositol-signaling molecules.

The Gram-negative marine bacterium *Vibrio parahaemolyticus* is a leading cause of gastroenteritis from the consumption of contaminated seafood (*1*). Many virulent strains of *V. parahaemolyticus* encode thermostable direct hemolysins and two type III secretion systems (T3SS1 and T3SS2) (*2*, *3*). The T3SS is a syringe-like mechanism often used by Gram-negative bacteria to introduce effector proteins into eukaryotic target cells during infection (*4*).

The *V. parahaemolyticus* T3SS1 injects effectors that cause a rapid, orchestrated cell death mediated by autophagy, cell rounding, and then cell lysis (*5*). One effector, VopQ, is both necessary and sufficient to induce autophagy (*6*), whereas another effector, VopS, is an AMPylator that contributes to cell rounding by modifying a conserved threonine residue on the Rho family of guanosine triphosphatases (GTPases) with adenosine 5'-monophosphate (AMP), preventing their interaction with downstream-signaling molecules (*7*). Upon analysis of the contribution of other T3SS1 effectors (*8*) involved in this paradigm of cell death, we found that a strain with only a functional T3SS1 (POR3) that was deleted for the effector VPA0450 (POR3Δ*vpa0450*) (Fig. 1B and fig. S1A) caused cell rounding faster than the parental POR3 strain or the complemented strain (POR3Δ*vpa0450* + VPA0450) during infection of HeLa cells (Fig. 1, A and C, respectively) (*9*). Additionally, both the POR3 strain (Fig. 1, E and I) and the complemented POR3Δ*vpa0450* + VPA0450 strain (Fig. 1, G and J) induced a transient blebbing of the host cell membrane before cell rounding, whereas POR3Δ*vpa0450* initiated rounding without any blebbing (Fig. 1, B and F). Further analysis revealed that POR3Δ*vpa0450* delayed lysis of the infected cell by approximate-

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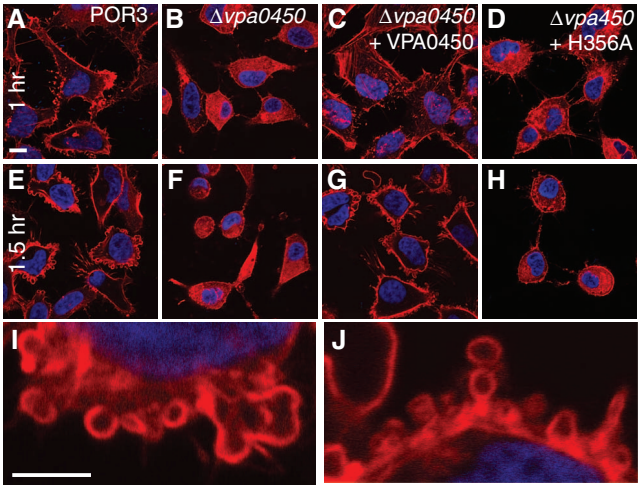


Fig. 1. Expression of VPA0450 leads to rapid host cell lysis. HeLa cells were infected with POR3, POR3Δ*vpa0450*, POR3Δ*vpa0450* + VPA0450, or POR3Δ*vpa0450* + VPA0450-H356A and visualized with confocal microscopy at, respectively, (**A** to **D**) 1 hour and (**E** to **H**) 1.5 hours. Scale bar, 10 μm. Blebbing is shown in detail from (**I**) POR3 and (**J**) POR3Δ*vpa0450* + VPA0450 infection at 1.5 hours. Scale bar, 5 μm. Actin cytoskeleton was stained with rhodamine-phalloidin (red), and nuclei were stained with Hoechst (blue). (**K**) Hela cells were infected with POR3 (circles), POR3Δ*vpa0450* (squares), or POR3Δ*vpa0450* + VPA0450 (triangles), and lactate dehydrogenase (LDH) release was evaluated as a measure of cytotoxicity and host cell lysis. Data are means ± SD ($n = 3$ samples) from a representative experiment repeated in triplicate.

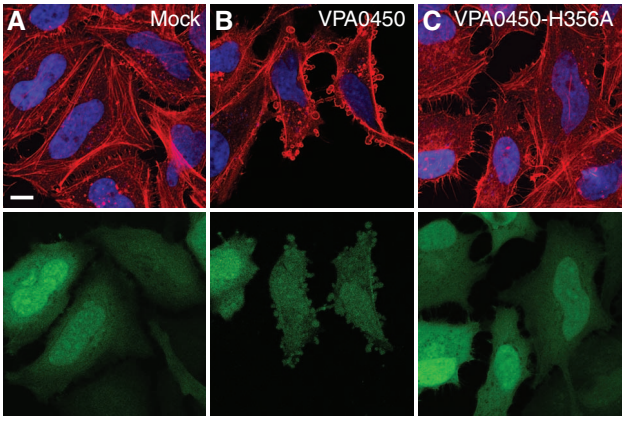
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ly 1 hour as compared with the POR3 strain (Fig. 1K). Complementation of the deleted strain with VPA0450 restored the lysis activity of this strain back to wild-type levels (Fig. 1K). Thus, the presence of VPA0450 contributed to a blebbing phenomenon during the early stages of infection and to efficient lysis during the later stages of infection.

Having correlated the presence of VPA0450 with efficient lysis of infected cells, we next investigated whether VPA0450 alone was sufficient to induce host cell lysis. Although HeLa cells transfected with empty vector exhibited the predicted morphology (Fig. 2A), cells transfected with VPA0450 did not lyse but remained attached to the plate and were covered by small membrane blebs that were consistent with those seen during infection (Fig. 2B). Blebs appeared not only on the surface of the cell but also on the surface of other blebs (movie S1). Thus, on its own VPA0450 did not directly lyse cells but did dramatically change the surface of transfected cells.

To investigate the activity that VPA0450 might contain to cause blebbing and facilitate lysis during infection, we performed bioinformatic analysis and discovered that VPA0450 has homology with effectors from other pathogenic bacteria, including *Aeromonas salmonicida* (Ati2, 87% identity), which is the causative agent of furunculosis in salmonid fish and of considerable importance to the fisheries industry (*10*), and the entemopathogenic nematode commensal *Photorhabdus luminescens* TTO1 (Plu4615, 93% identity). These effectors are most similar to proteins in the endonuclease/exonuclease/phosphatase family of eukaryotic proteins (fig. S2). VPA0450 contains five of the six catalytic motifs found in the active site for the family of inositol polyphosphate 5-phosphatases (IPP5Cs), enzymes that specifically dephosphorylate the D5 phosphate on an inositol ring (fig. S3, A and B) (*11*, *12*).

Disrupting the homeostasis of phosphatidylinositols in a cell can result in a wide range of



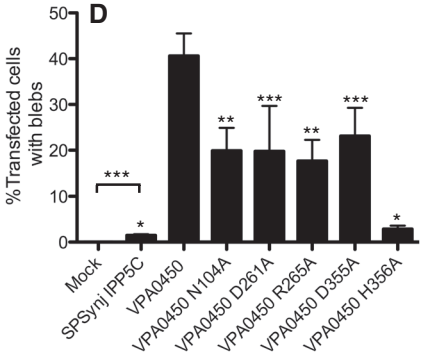
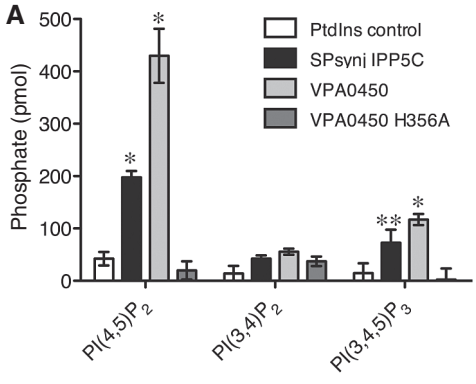
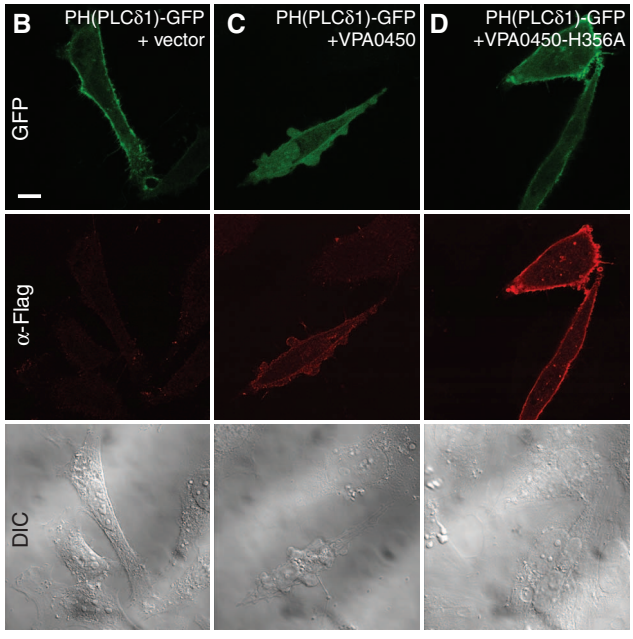


Fig. 2. VPA0450 mimics the synaptojanin inositol polyphosphate 5-phosphatase catalytic domain. HeLa cells were transfected with pEGFP-N1 and (**A**) pSFFV empty vector (**B**) pSFFV-VPA0450-flag, or (**C**) pSFFV-VPA0450-H356A-flag and visualized with confocal microscopy. Expression of GFP (green) denotes transfected cells, actin cytoskeleton stained with rhodamine-phalloidin (red), and nuclei stained with Hoechst (blue). Scale bar, 10 μm. (**D**) Quantitation of blebbing in Hela cells transfected with empty vector, SPsynj IPP5C, VPA0450, or VPA0450 point mutants. Data are means ± SD from three independent experiments. Asterisks refer to statistically significant differences between VPA0450-transfected cells and the other constructs (*$P < 0.001$, **$P < 0.01$, ***$P < 0.05$, $n = 3$ independent experiments) using a pairwise, two-tailed *t*-test.





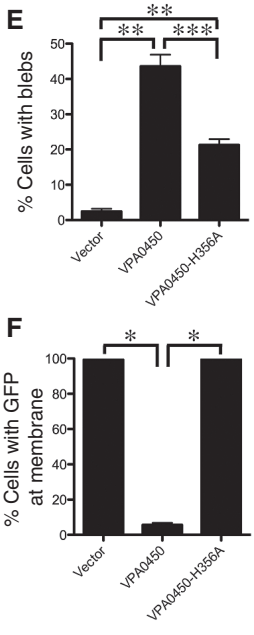


Fig. 3. VPA0450 has inositol polyphosphate 5-phosphatase activity. (**A**) Phosphatase activity of rSPsynj IPP5C, rVPA0450, and rVPA0450-H356A on phosphatidylinositol (4,5) bisphosphate di-C_8 [PI(4,5)P_2], phosphatidylinositol (3,4) bisphosphate di-C_8 [PI(3,4)P_2], and phosphatidylinositol (3,4,5) triphosphate di-C_8 [PI(3,4,5)P_3], measured by means of malachite green assay of inorganic phosphate release. Asterisks refer to statistically significant differences between PtdIns control and each recombinant protein for a given substrate (*$P < 0.001$, **$P < 0.01$, $n = 3$ independent experiments) using a pairwise, two-tailed *t*-test. HeLa cells transfected with (**B**) PH(PLCδ1)-GFP and empty vector, (**C**) pSFFV-VPA0450-flag, or (**D**) pSFFV-VPA0450-H356A-flag and visualized by means of confocal microscopy. Green denotes expression of PH(PLCδ1)-GFP, orange denotes expression of VPA0450-flag, and grayscale denotes all cells. Scale bar, 10 μm. Quantitation of (**E**) blebbing or (**F**) GFP at the membrane in Hela cells transfected with 2xFYVE domain and empty vector, VPA0450, or VPA0450-H356A. Data are means ± SD from three independent experiments. Asterisks refer to statistically significant differences between VPA0450 transfected cells and the other constructs (*$P < 0.001$; **$P < 0.01$; ***$P < 0.05$, $n = 3$ independent experiments) using a pairwise, two-tailed *t*-test.

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consequences, including the promotion of tumor progression and disruption of normal neuronal development (*11*–*13*). Synaptojanin from the fission yeast *Schizosaccharomyces pombe* (SPsynj) is a multi-domain protein containing an IPP5C domain that is associated with the regulation of intracellular calcium signaling, actin cytoskeleton, and cell growth and motility (*11*, *14*, *15*). This domain is different from the synaptojanin Sac1 domain that is homologous to *Salmonella* effectors SigD and SopB, which are necessary for phagocytosis as well as sustaining the *Salmonella*-containing vacuole during intracellular infection by that pathogen (fig. S3B) (*16*). Overexpression of membrane-associated Inp54p, an SPsynj IPP5C domain homolog, induces cellular blebbing (*14*). Expression of the SPsynj IPP5C domain in HeLa cells also resulted, albeit to a lesser extent, in cellular blebbing (fig. S3C) similar to that seen for VPA0450 (Fig. 2B). The observation that VPA0450 causes a more dramatic phenotype as compared with that of an endogenous eukaryotic homolog is characteristic of T3SS effectors. Thus, VPA0450 might contain an inositol polyphosphate 5-phosphatase activity that can alter membrane dynamics in infected and transfected cells.

Structural analysis of this family of D5-phosphatases revealed a catalytic pocket similar to that of apurinic/apyrimidinic endonucleases that uses a histidine as a base for catalysis. To test whether the conserved histidine in the putative catalytic site of VPA0450 was important for activity, we transfected the mutant VPA0450-H356A into HeLa cells. No substantial morphological changes were observed in cells as compared with those from vector control transfected cells (Fig. 2C). Complementation of the POR3Δ*vpa0450* strain with VPA0450-H356A did not rescue the defect in blebs or the delayed cytotoxicity (Fig. 1, D and H, and fig. S1). On the basis of this finding, we went further to ask whether other residues in the putative catalytic pocket might contribute to the activity of VPA0450. Mutation of amino acids that are predicted to coordinate substrate binding and facilitate catalysis resulted in enzymes that were less effective at inducing cellular blebbing (Fig. 2D and fig. S3, D to H). Thus, VPA0450 might be disrupting homeostasis in the infected cell by disrupting phosphatidylinositol signaling.

To directly test for putative phosphatidylinositol phosphatase activity, we performed a malachite green assay (*17*) with recombinant proteins, including VPA0450, VPA0450-H356A, and SPSynj IPP5C, so as to measure phosphate release. rVPA0450 and rSPSynj IPP5C, but not mutant rVPA0450-H356A, hydrolyzed phosphate off phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2] (Fig. 3A). None of the recombinant enzymes hydrolyzed phosphate off of phosphatidylinositol 3,4-bisphosphate (Fig. 3A). Both rVPA0450 and rSPSynj IPP5C, but not mutant rVPA0450-H356A, cleaved phosphate off phosphatidylinositol 3,4,5-triphosphate (Fig. 3A). Thus, rVPA0450 contained phosphatidylinositol phosphatase activity that specifically hydrolyzed the D5 inositol phosphate.

The plasma membrane PtdIns(4,5)P_2 mediates signaling between the plasma membrane and the actin cytoskeleton by acting as a docking site for adaptor molecules. The pleckstrin homology (PH) domain from phospholipase-Cδ1 [PH(PLCδ1)] binds PtdIns(4,5)P_2 to tether the actin cytoskeletal machinery to the membrane. Reversible inositol-mediated tethering and changes in association with the actin cortex permits membranes to swell, promoting cell movement (*14*, *18*). We predicted that the constitutive hydrolysis of PtdIns(4,5)P_2 by VPA0450 would result in unregulated membrane blebbing because PH(PLCδ1) domain–like proteins could no longer dock efficiently at the membrane. To test this hypothesis, we transfected Hela cells with a PH(PLCδ1)–green fluorescent protein (GFP) domain in the presence of empty vector, VPA0450, or VPA0450-H356A (Fig. 3, B to F, and fig. S4A). In cells cotransfected with either empty vector or VPA0450-H356A, we observed GFP-mediated fluorescence around the edge of the cell, whereas in the presence of wild-type VPA0450 the GFP fluorescence appeared cytoplasmic, indicating that VPA0450 and its IPP5C activity disrupted the proper association of the PH(PLCδ1) domain with the membrane. HeLa cells infected with either POR3 or POR3Δ*vpa0450* + VPA0450, but not POR3Δ*vpa0450*, did not show localization of PtdIns(4,5)P_2 at plasma membrane when cells began to bleb and round (fig. S4, B to E). 2xFYVE-GFP binds specifically to phosphatidylinositol 3-phosphate enriched on endosomes (*19*). Cells cotransfected with 2xFYVE-GFP and empty vector, VPA0450, or VPA0450-H356A all resulted in cytoplasmic GFP punctae that is consistent with endosomal staining, indicating that VPA0450 does not affect the inositol D3 phosphate (fig. S5, A to F). Thus, VPA0450 can act to hydrolyze PtdIns(4,5)P_2 specifically and destroy docking sites for PtdIns(4,5)P_2–specific PH domains, disrupting the interaction between the actin cytoskeleton and the membrane.

Bacterial pathogens use many tools to disrupt the actin cytoskeleton in an infected cell, including GTP-activating proteins and exchange factors, kinases, AMPylators, and phosphatases (*7*, *20*, *21*). *V. parahaemolyticus* uses a distinct repertoire of effectors during infection to unregulate autophagy, then cause cell rounding, followed by cell lysis. In an uninfected cell, integrity of the plasma membrane is maintained through the dynamic interaction of the cytoskeleton with various actin-binding and -nucleating proteins (fig. S6A). We have shown that transfection of VPA0450 into HeLa cells is sufficient to form membrane blebs similar to those seen during infection by *V. parahaemolyticus* carrying the *vpa0450* gene but is not able to directly cause cell lysis (fig. S6B). We propose that VPA0450 facilitates cell lysis by using its IPP5C domain to hydrolyze PtdIns(4,5)P_2, compromising the integrity of the cell membrane during late stages of infection (fig. S6C). The target of VPA0450, the D5 phosphate on the inositol ring, is critical to mediate a conversation between the actin cytoskeleton and the cell membrane. Disruption of this dynamic process by VPA0450, coupled with the activities of other effectors, including VopQ and VopS, leads to destabilization of the actin cytoskeleton and rapid cell lysis during infection. The discovery of the activity for VPA0450 highlights a lipid target that is crucial for cellular movement that bacterial pathogens can exploit to disrupt the integrity of the cell membrane and its dynamic interaction with the actin cytoskeleton.

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Supporting Online Material

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α-Synuclein Promotes SNARE-Complex Assembly in Vivo and in Vitro

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Presynaptic nerve terminals release neurotransmitters repeatedly, often at high frequency, and in relative isolation from neuronal cell bodies. Repeated release requires cycles of soluble *N*-ethylmaleimide–sensitive factor attachment protein receptor (SNARE)–complex assembly and disassembly, with continuous generation of reactive SNARE-protein intermediates. Although many forms of neurodegeneration initiate presynaptically, only few pathogenic mechanisms are known, and the functions of presynaptic proteins linked to neurodegeneration, such as α-synuclein, remain unclear. Here, we show that maintenance of continuous presynaptic SNARE-complex assembly required a nonclassical chaperone activity mediated by synucleins. Specifically, α-synuclein directly bound to the SNARE-protein synaptobrevin-2/vesicle-associated membrane protein 2 (VAMP2) and promoted SNARE-complex assembly. Moreover, triple-knockout mice lacking synucleins developed age-dependent neurological impairments, exhibited decreased SNARE-complex assembly, and died prematurely. Thus, synucleins may function to sustain normal SNARE-complex assembly in a presynaptic terminal during aging.

In presynaptic terminals, neurotransmitter release requires a tightly coordinated membrane fusion machinery whose central components are soluble *N*-ethylmaleimide–sensitive factor (NSF) attachment protein receptor (SNARE) and Sec1/Munc18–like proteins (*1*–*3*). Terminals release neurotransmitters thousands of times per minute; during each release reaction, SNARE-complex assembly and disassembly generates highly reactive unfolded SNARE protein intermediates, rendering the terminals potentially vulnerable to activity-dependent degeneration. Much evidence points to presynaptic terminals as an initiation site for neurodegeneration (*4*–*6*), and knockout (KO) of at least one presynaptic chaperone protein, cysteine string protein-α (CSPα), causes fulminant neurodegeneration in mice (*7*). Synucleins are abundant presynaptic proteins that are expressed from three genes (α-, β-, and γ-synuclein) (*8*). α-Synuclein is involved in neurodegeneration (*9*–*11*), and γ-synuclein may contribute to pro-

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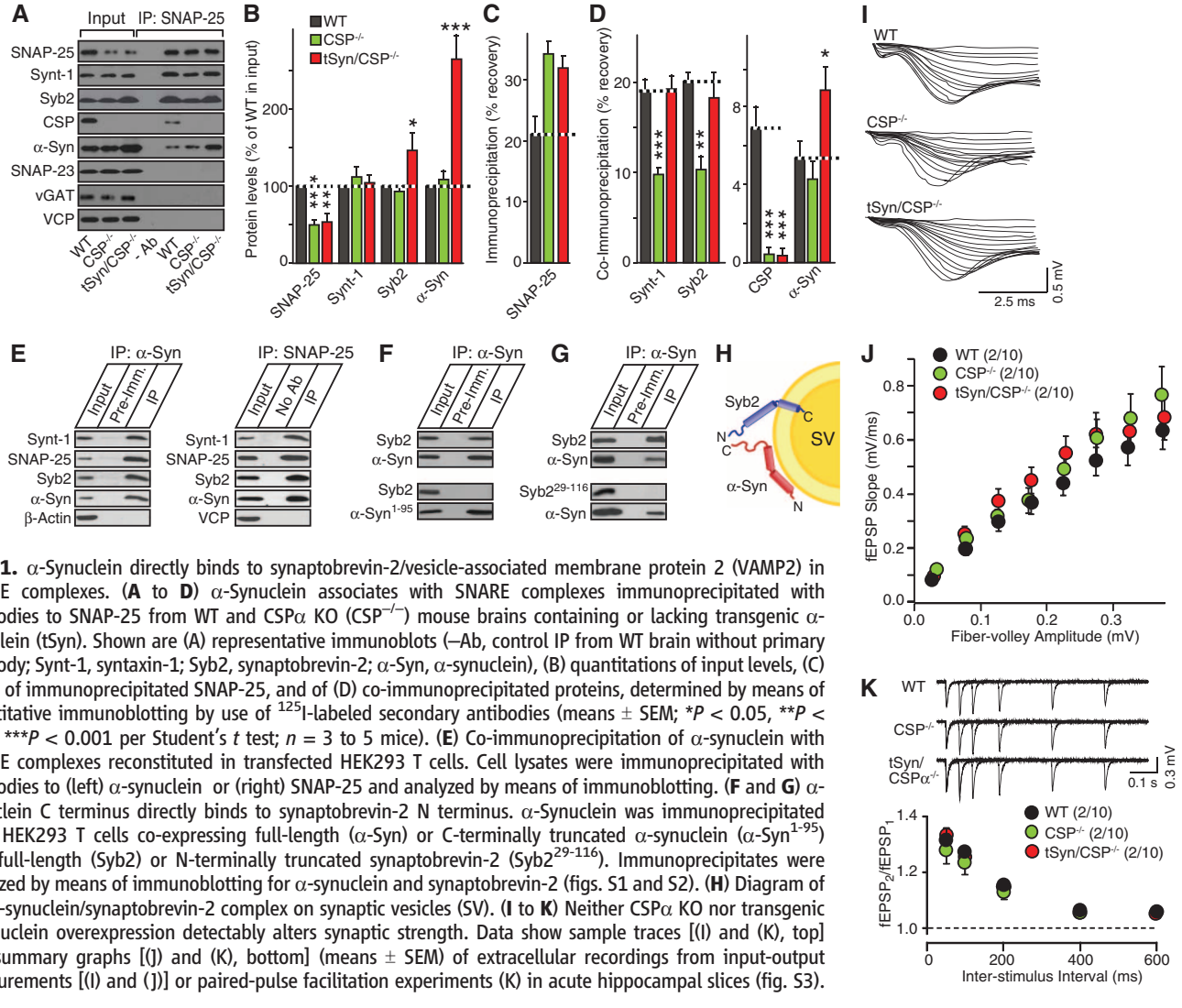


Fig. 1. α-Synuclein directly binds to synaptobrevin-2/vesicle-associated membrane protein 2 (VAMP2) in SNARE complexes. (**A** to **D**) α-Synuclein associates with SNARE complexes immunoprecipitated with antibodies to SNAP-25 from WT and CSPα KO ($CSP^{-/-}$) mouse brains containing or lacking transgenic α-synuclein (tSyn). Shown are (A) representative immunoblots (–Ab, control IP from WT brain without primary antibody; Synt-1, syntaxin-1; Syb2, synaptobrevin-2; α-Syn, α-synuclein), (B) quantitations of input levels, (C) levels of immunoprecipitated SNAP-25, and of (D) co-immunoprecipitated proteins, determined by means of quantitative immunoblotting by use of ^{125}I-labeled secondary antibodies (means ± SEM; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ per Student's *t* test; $n = 3$ to 5 mice). (**E**) Co-immunoprecipitation of α-synuclein with SNARE complexes reconstituted in transfected HEK293 T cells. Cell lysates were immunoprecipitated with antibodies to (left) α-synuclein or (right) SNAP-25 and analyzed by means of immunoblotting. (**F** and **G**) α-Synuclein C terminus directly binds to synaptobrevin-2 N terminus. α-Synuclein was immunoprecipitated from HEK293 T cells co-expressing full-length (α-Syn) or C-terminally truncated α-synuclein (α-Syn^{1-95}) with full-length (Syb2) or N-terminally truncated synaptobrevin-2 ($Syb2^{29-116}$). Immunoprecipitates were analyzed by means of immunoblotting for α-synuclein and synaptobrevin-2 (figs. S1 and S2). (**H**) Diagram of the α-synuclein/synaptobrevin-2 complex on synaptic vesicles (SV). (**I** to **K**) Neither CSPα KO nor transgenic α-synuclein overexpression detectably alters synaptic strength. Data show sample traces [(I) and (K), top] and summary graphs [(J) and (K), bottom] (means ± SEM) of extracellular recordings from input-output measurements [(I) and (J)] or paired-pulse facilitation experiments (K) in acute hippocampal slices (fig. S3).

gression of many types of cancer (*12*). Synucleins may modify neurotransmitter release (*13*, *14*), but their physiological functions remain unknown. Transgenic expression of α-synuclein abolishes the lethal neurodegeneration induced by KO of CSPα, whereas deletion of endogenous synucleins accelerates this neurodegeneration (*15*). Mice in which CSPα has been knocked out (CSPα KO mice) exhibit decreased levels of the SNARE protein SNAP-25 and impaired SNARE-complex assembly, suggesting a link between SNARE-complex assembly and neurodegeneration. α-Synuclein rescues SNARE-complex assembly but not SNAP-25 levels (*15*). This result indicates that α-synuclein may enhance SNARE-protein function and hence compensate for the CSPα deletion. To address this hypothesis, we examined the role of α-synuclein in SNARE-complex assembly and in the maintenance of continuous SNARE-cycling in presynaptic terminals over the lifetime of an animal.

We immunoprecipitated SNARE complexes from brain homogenates using antibodies to SNAP-25. We measured the levels of SNARE proteins and α-synuclein in the input and immunoprecipitates from littermate wild-type (WT) mice, CSPα KO mice, and CSPα KO mice rescued with transgenic α-synuclein (*16*). As expected (*15*), transgenic α-synuclein rescued the SNARE-complex assembly deficit but not the ~50% decrease in SNAP-25 levels in CSPα KO mice (Fig. 1, A to D). Unexpectedly, however, endogenous and transgenic α-synuclein were co-immunoprecipitated with SNAP-25, independently of CSPα (Fig. 1, A to D, and fig. S1, A to C). Moreover, α-synuclein co-immunoprecipitated SNARE proteins from mouse brains (fig. S1, D and E) or when α-synuclein and SNARE proteins were co-expressed in human embryonic kidney (HEK) 293 cells (Fig. 1E). When each SNARE protein was co-expressed separately with α-synuclein (Fig. 1, F and G, and fig. S1, F to H), only synaptobrevin-2 was co-immunoprecipitated with α-synuclein. Direct α-synuclein–binding to synaptobrevin-2 was confirmed through glutathione *S*-transferase fusion protein pulldowns (fig. S1I) and liposome-binding experiments (fig. S2). Removal of either the C-terminal 44 residues from α-synuclein—which are not involved in phospholipid binding (figs. S1J and S1K) (*17*)—or deletion of the N-terminal 28 residues from synaptobrevin-2—which are not involved in SNARE-complex formation (*1*–*3*)—blocked α-synuclein binding to synaptobrevin-2 (Fig. 1, F and G). Thus, the C terminus of α-synuclein

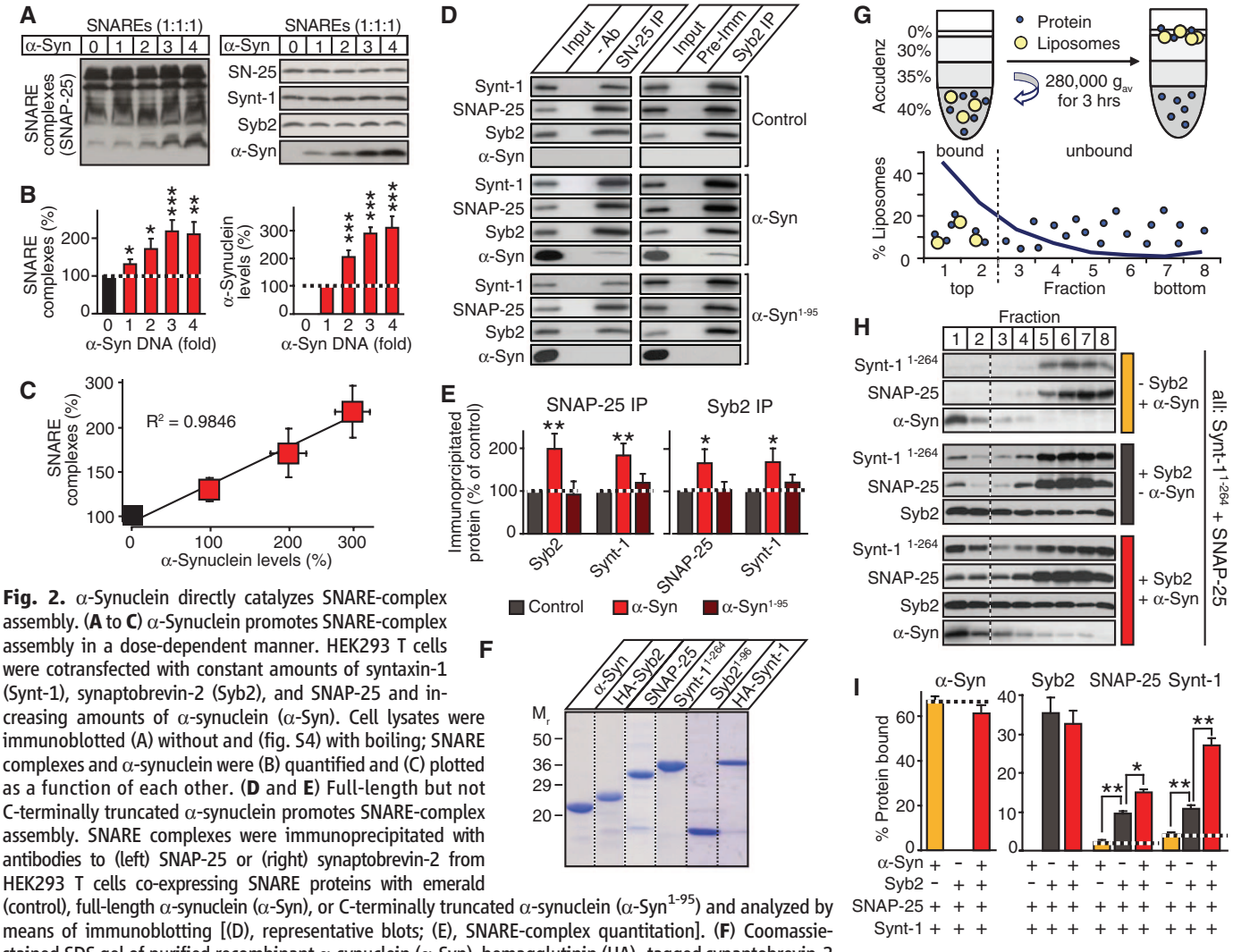


Fig. 2. α-Synuclein directly catalyzes SNARE-complex assembly. (**A** to **C**) α-Synuclein promotes SNARE-complex assembly in a dose-dependent manner. HEK293 T cells were cotransfected with constant amounts of syntaxin-1 (Synt-1), synaptobrevin-2 (Syb2), and SNAP-25 and increasing amounts of α-synuclein (α-Syn). Cell lysates were immunoblotted (A) without and (fig. S4) with boiling; SNARE complexes and α-synuclein were (B) quantified and (C) plotted as a function of each other. (**D** and **E**) Full-length but not C-terminally truncated α-synuclein promotes SNARE-complex assembly. SNARE complexes were immunoprecipitated with antibodies to (left) SNAP-25 or (right) synaptobrevin-2 from HEK293 T cells co-expressing SNARE proteins with emerald (control), full-length α-synuclein (α-Syn), or C-terminally truncated α-synuclein (α-Syn$^{1-95}$) and analyzed by means of immunoblotting [(D), representative blots; (E), SNARE-complex quantitation]. (**F**) Coomassie-stained SDS gel of purified recombinant α-synuclein (α-Syn), hemagglutinin (HA)–tagged synaptobrevin-2 (HA-Syb2), SNAP-25, C-terminally truncated syntaxin-1 (Synt-1$^{1-264}$) or synaptobrevin-2 (Syb2$^{1-96}$), and full-length HA-tagged syntaxin-1 (HA-Synt-1). (**G**) In vitro SNARE-complex assembly assay. Liposomes containing or lacking reconstituted synaptobrevin-2 are mixed with SNAP-25 and C-terminally truncated syntaxin-1, with or without α-synuclein, and floated by means of density gradient centrifugation. SNARE-complex assembly is measured as coflotation of SNAP-25 and syntaxin-1 with liposomes in the top two fractions. (**H** and **I**) Distribution of SNAREs and α-synuclein in liposome flotation gradients, shown as (H) representative immunoblots and (I) quantitations of liposome-bound proteins. Whereas α-synuclein (α-Syn) and synaptobrevin-2 are directly liposome-bound, SNAP-25 and syntaxin-1 (Synt-1) only bind to liposomes via SNARE-complex formation. Data are means ± SEM (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ by Student's *t* test; $n = 3$ to 8 independent experiments).

binds to the N terminus of synaptobrevin-2 on the synaptic vesicle surface (Fig. 1H).

How does binding of α-synuclein to SNARE complexes relate to its ability to rescue the neurodegeneration of CSPα KO mice? The activity-dependence of neurodegeneration in CSPα KO mice (*7*, *18*) suggests that α-synuclein may indirectly rescue the CSPα KO phenotype by inhibiting synaptic strength. However, electrophysiological measurements in acute brain slices from WT and CSPα KO mice, without or with transgenic α-synuclein expression, failed to reveal changes in synaptic strength (Fig. 1, I to K, and fig. S3). As an alternative hypothesis, we thus tested whether α-synuclein directly rescued the neurodegeneration of CSPα KO mice by promoting SNARE-complex assembly.

We co-transfected HEK293 cells with constant amounts of SNARE protein and increasing amounts of α-synuclein expression vectors and measured SNARE-complex assembly as a function of α-synuclein, using immunoblotting of nonboiled samples as an assay (Fig. 2, A and B) (*19*). We observed a linear relationship between SNARE-complex assembly and α-synuclein levels (Fig. 2C and fig. S4). Using co-immunoprecipitation assays, we confirmed that full-length but not C-terminally truncated α-synuclein promoted SNARE-complex assembly (Fig. 2, D and E, and figs. S4 and S5).

Because the effect of α-synuclein in transfected HEK293 cells may have been indirect, we examined the function of α-synuclein in a purely in vitro system. We reconstituted recombinant synaptobrevin-2 into liposomes (fig. S2, A to C) and measured SNARE-complex assembly with soluble recombinant syntaxin-1 and SNAP-25 onto these liposomes as a function of α-synuclein (Fig. 2, F to I). SNARE-complex assembly was monitored by means of flotation of liposomes on an Accudenz centrifugation gradient (Accurate Chemical & Scientific Corporation, Westbury, New York) (Fig. 2G). Reconstituted and free synaptobrevin-2 were not separated before the assay; as a result, synaptobrevin-2 was distributed across the gradient, whereas α-synuclein—which quantitatively binds to phospholipids (*17*)—was exclusively present in the bound fractions at the top of the gradient (Fig. 2H). In this purified system, SNARE-complex assembly was strongly enhanced by α-synuclein (Fig. 2I).

To assess the physiological importance of the observed biochemical activity of α-synuclein, we generated αβγ-synuclein triple knockout (TKO) mice. Similar to single and double α-, β-, and γ-

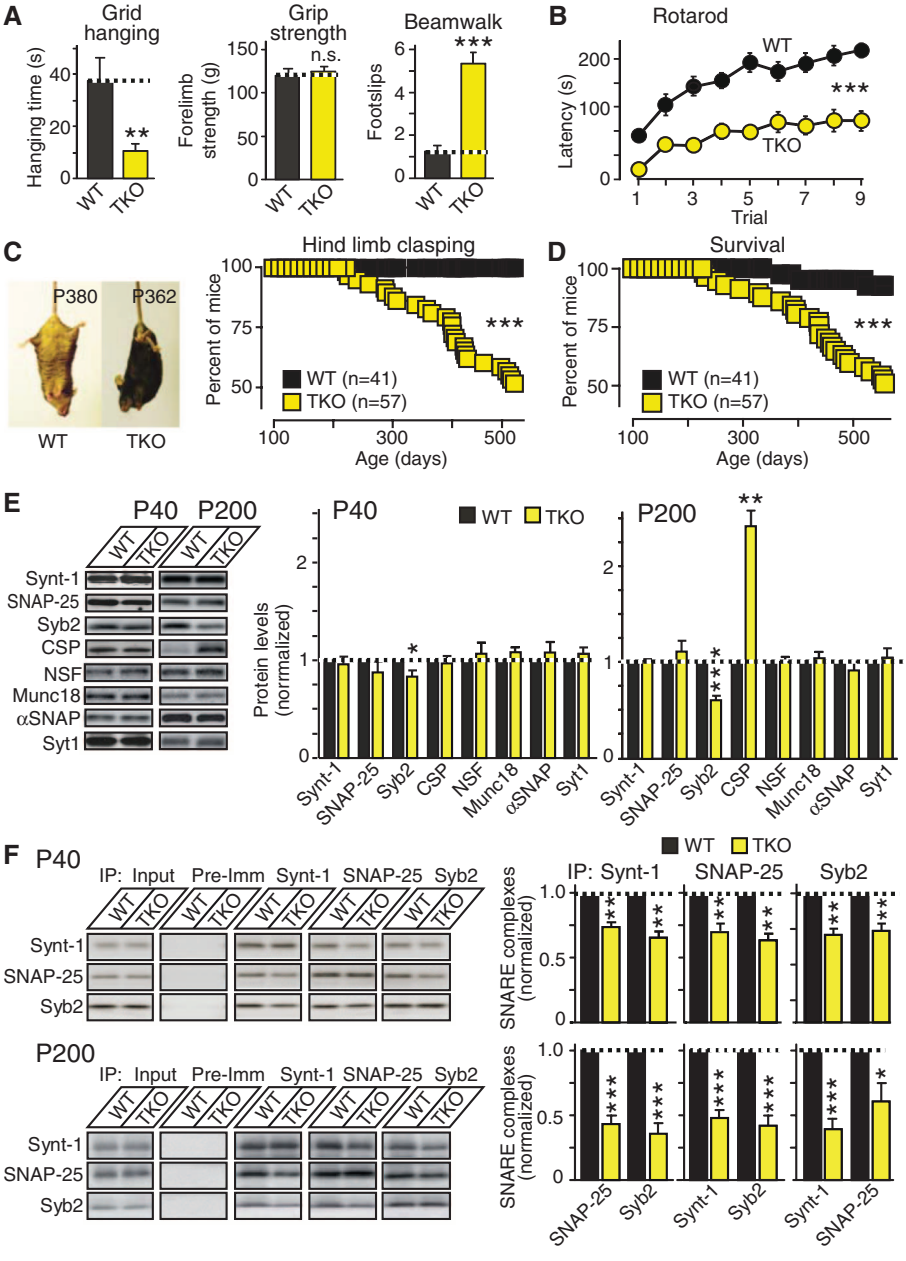


Fig. 3. αβγ-Synuclein TKO mice exhibit impaired survival and decreased SNARE-complex assembly. (**A** and **B**) Neurological impairments in αβγ-synuclein TKO mice (at P300). Data show (A) latency to fall off an inverted wire grid (grid-hanging time; WT, n = 6 mice; TKO, n = 7 mice), forelimb grip strength (measured using a digital grip strength meter; WT, n = 9 mice; TKO, n = 11 mice), and footslips during beamwalking (WT, n = 9 mice; TKO, n = 11 mice) and (B) the latency to fall off an accelerating rotarod (5-min trials; WT, n = 9 mice; TKO, n = 11 mice). (**C**) Hind-limb clasping of WT and TKO mice as a function of age (left, representative images; right, clasping incidence; WT, n = 41 mice; TKO, n = 57 mice). (**D**) Age-dependent mortality of WT and TKO mice (WT, n = 41 mice; TKO, n = 57 mice). (**E**) Age-dependent changes in protein levels in TKO mice (left, representative blots; right, quantitations). (**F**) SNARE-complex assembly analyzed by means of co-immunoprecipitation of SNAREs in brain lysates from WT and TKO mice at P40 (top) and P200 (bottom). (Left) Representative blots. (Right) Quantitations from multiple independent experiments (n = 3 to 5 mice; Syb2, synaptobrevin-2; Synt-1, syntaxin-1). See figs. S6 to S9 for further data. Data are means ± SEM, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ by [(A), (E), and (F)] Student's t test, (B) two-way analysis of variance (ANOVA), or (C) Mantel-Cox test.

synuclein KO mice (*20*–*22*), young TKO mice displayed no obvious phenotype. During aging, however, TKO mice developed severe neurological impairments (Fig. 3, A to C) and died prematurely (Fig. 3D). Although TKO mice did not display overt neurodegeneration or changes in synaptic strength (figs. S6 and S7), and most synaptic proteins were unchanged, we observed a marked, age-dependent decrease in synaptobrevin-2 and a large increase in CSPα levels (Fig. 3E and fig. S8), which is consistent with their functional interaction. Noticeably, TKO mice exhibited an age-dependent decrease in SNARE-complex assembly (Fig. 3F and fig. S9). Thus, synucleins are required for maintaining normal SNARE-complex assembly during aging in mice.

We next examined whether the SNARE-complex assembly deficit in TKO mice could be reversed by the reintroduction of α-synuclein. Expression of increasing amounts of α-synuclein in cultured TKO neurons produced a corresponding dose-dependent increase in SNARE-complex assembly (Fig. 4A) similar to the HEK293 cell experiments (Fig. 2, A to C). The C terminus of α-synuclein that bound to synaptobrevin-2 (Fig. 1) was required for its promotion of SNARE-complex assembly (Fig. 4B), even though it was not required for synaptic targeting of α-synuclein (fig. S10), confirming that α-synuclein acts by binding to synaptobrevin-2.

The age-dependent impairment of SNARE-complex assembly in TKO mice suggests an activity-dependent phenotype. To test this hypothesis, we blocked or stimulated synaptic activity in cultured TKO and WT neurons for 36 hours using tetrodotoxin (TTX, an inhibitor of voltage-gated Na^+-channels) or increased ambient Ca^{2+} concentrations (4 mM). Cultured TKO neurons exhibited a significant SNARE-complex assembly deficit that was dramatically enhanced by increased synaptic activity but rescued by decreased synaptic activity (Fig. 4C and fig. S11). The activity-dependent phenotype in TKO neurons developed in a time-dependent manner and was rescued by WT but not C-terminally truncated α-synuclein (Fig. 4D and fig. S11C), which is consistent with the biochemical activity of α-synuclein (Fig. 2).

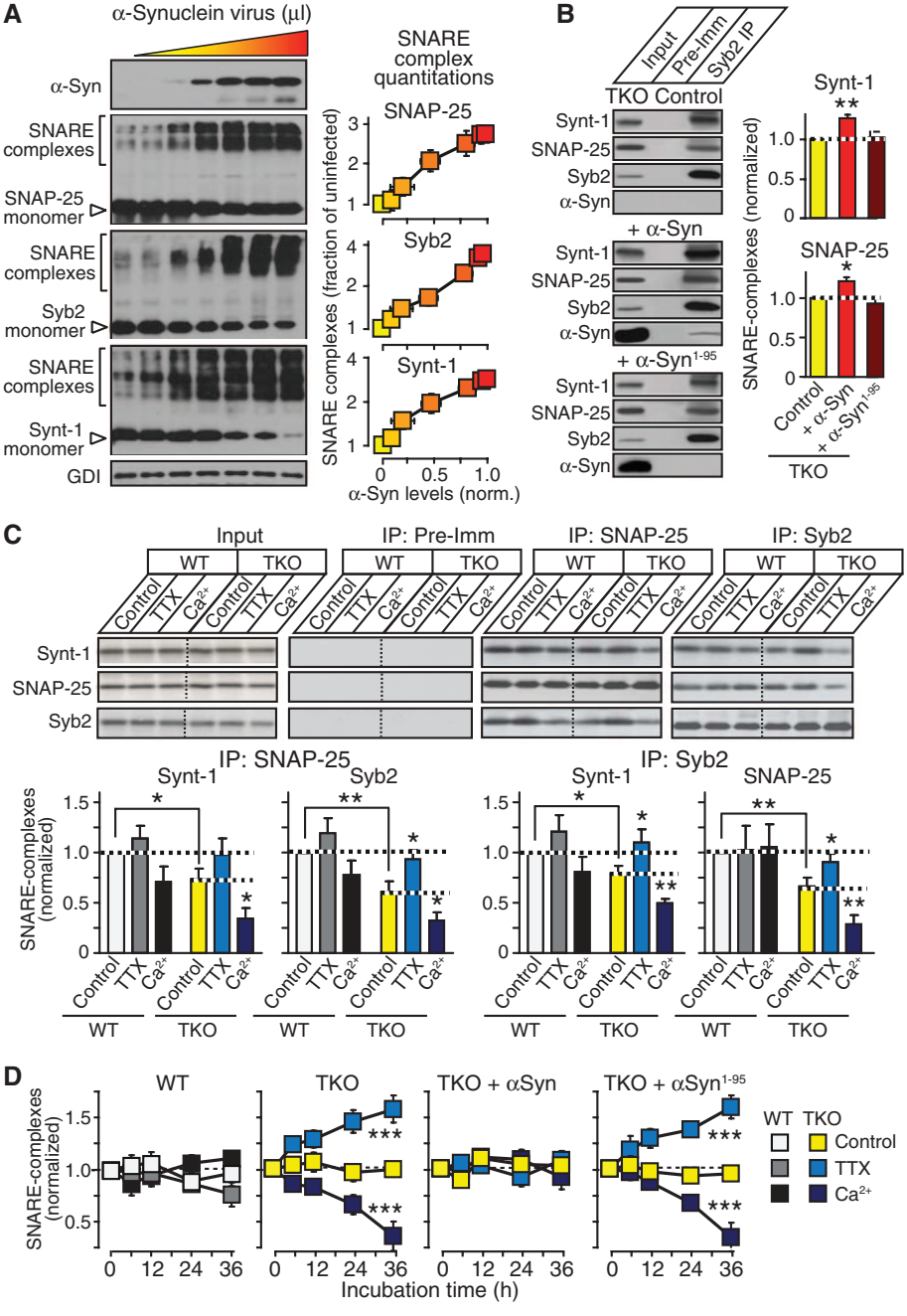


Fig. 4. α-Synuclein boosts SNARE-complex assembly during synaptic activity. (**A**) Linear relationship between α-synuclein levels and SNARE-complex assembly in αβγ-synuclein TKO neurons infected at DIV4 with increasing amounts of lentivirus expressing α-synuclein and analyzed by means of immunoblotting of nonboiled samples at DIV17 (n = 3 to 6 cultures). (**B**) Full-length (α-Syn) but not truncated α-synuclein (α-Syn^{1-95}) enhances SNARE-complex assembly in TKO neurons, as measured by means of co-immunoprecipitation of syntaxin-1 (Synt-1) and SNAP-25 with synaptobrevin-2 (Syb2) (control is plain virus; left, representative blots; right, quantitations; n = 3 to 6 cultures). (**C**) Activity-dependence of SNARE-complex assembly in cultured WT and TKO neurons. Neurons were incubated at DIV10 for 36 hours in control medium, 0.5 μM tetrodotoxin (TTX), or 4 mM Ca^{2+} (Ca^{2+}). SNARE-complex assembly was measured with co-immunoprecipitation of SNARE proteins with SNAP-25 and synaptobrevin-2. Representative blots are shown at top (Pre-Imm, pre-immune control), and quantitations from multiple independent experiments are shown at bottom (n = 3 to 6 cultures). (**D**) Time course of activity-induced changes in SNARE-complex assembly in WT and TKO neurons and in TKO neurons infected with lentiviruses expressing full-length (α-Syn) or C-terminally truncated α-synuclein (α-Syn^{1-95}). Neurons were incubated in control medium, 0.5 μM TTX, or 4 mM Ca^{2+} for the indicated times, and SNARE-complex assembly was measured with immunoblotting for SNAP-25 in unboiled samples (n = 3 cultures). See figs. S10 and S11 for further data. Data are means ± SEM, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ by [(A) to (C)] Student's t test or (D) one-way ANOVA.

We found that α-synuclein directly promotes SNARE-complex assembly through a nonenzymatic mechanism that involves simultaneous binding of α-synuclein to phospholipids via its N terminus and to synaptobrevin-2 via its C terminus (fig. S12). The SNARE complex–promoting function of α-synuclein becomes important during increased synaptic activity and aging, rendering its action akin to a proofreading activity that is essential for the continued maintenance of SNARE-mediated fusion over the lifetime of an animal. As a result, loss of synuclein function manifests in an age-dependent loss of neuronal function, as revealed in αβγ-synuclein TKO mice whose phenotype resembles the delayed onset and slow progression of a neurodegenerative disease. Relative loss of α-synuclein function, for example by sequestration of α-synuclein in Lewy bodies or by increased truncation of α-synuclein during aging (*23*–*27*), may thus contribute to neurodegenerative diseases such as Parkinson's disease and Lewy body dementia. Furthermore, the increase in γ-synuclein in many cancers (*12*) may support enhanced membrane traffic in transformed cells.

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Materials and Methods
Figs. S1 to S12
References

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Stability of the Regulatory T Cell Lineage in Vivo

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Tissue maintenance and homeostasis can be achieved through the replacement of dying cells by differentiating precursors or self-renewal of terminally differentiated cells or relies heavily on cellular longevity in poorly regenerating tissues. Regulatory T cells (T_{reg} cells) represent an actively dividing cell population with critical function in suppression of lethal immune-mediated inflammation. The plasticity of T_{reg} cells has been actively debated because it could factor importantly in protective immunity or autoimmunity. By using inducible labeling and tracking of T_{reg} cell fate in vivo, or transfers of highly purified T_{reg} cells, we have demonstrated notable stability of this cell population under physiologic and inflammatory conditions. Our results suggest that self-renewal of mature T_{reg} cells serves as a major mechanism of maintenance of the T_{reg} cell lineage in adult mice.

Continuous replacement of differentiated cells within some tissues, such as the gut epithelium, is achieved by their de novo generation from a pool of precursor cells (*1*), whereas other tissues with reduced regenerative and proliferative capacity, such as the central nervous system, largely rely on cellular longevity for maintenance. Replication and self-renewal of differentiated cells, as shown for pancreatic β-cells, represents another potential mechanism underlying tissue maintenance (*2*). The latter strategy raises the issue of stability versus plasticity of a differentiated state, which is of particular importance for the proliferating cells of the immune system. Regulatory T cells (T_{reg} cells), defined by expression of the transcription factor Foxp3, represent a dedicated suppressor cell type whose maintenance is required throughout life to restrain fatal autoimmunity (*3*–*7*). The instability of the T_{reg} cell fate—their ability to further differentiate into diverse effector T cell (T_{eff} cell) types—remains controversial. Many T_{reg} cells express T cell receptors (TCRs) with heightened affinity for self-peptide major histocompatibility complexes (MHCs) (*8*–*11*), and ablation of a conditional *Foxp3* allele in mature T_{reg} cells results in generation of "former" T_{reg} cells that are capable of causing inflammatory tissue lesions (*12*). Thus, the stability of Foxp3 expression under basal and inflammatory conditions is an important determinant of immune homeostasis and of immediate relevance to the question of whether T_{reg} cells represent a distinct, stable cell lineage or a transient meta-stable activation state. Genetic fate mapping provides means to evaluate both lineage stability and informs mechanisms of tissue maintenance in vivo.

We used this approach to evaluate T_{reg} cell lineage stability under basal and inflammatory conditions and generated knock-in mice harboring a cassette containing an internal ribosome entry site (IRES) followed by DNA sequence encoding a "triple" fusion protein of the enhanced green fluorescent protein (eGFP) with Cre recombinase and mutated human estrogen receptor ligand-binding domain (ERT2) inserted into the 3′ untranslated region (UTR) of the *Foxp3* gene (*13*). The *Foxp3*$^{eGFP\text{-}Cre\text{-}ERT2}$ mice were bred to mice that expressed a Cre recombination reporter allele of the ubiquitously expressed *ROSA26* locus containing a loxP site–flanked STOP cassette followed by a DNA sequence encoding yellow fluorescent protein (YFP) (R26Y) (fig. S1). In the *Foxp3*$^{eGFP\text{-}Cre\text{-}ERT2}$ × R26Y mice, the GFP-CreERT2 fusion protein is sequestered in the cytosol, and therefore YFP is not expressed; but treatment with tamoxifen allows for nuclear translocation of the fusion protein, excision of the floxed STOP cassette, and constitutive and heritable expression of YFP in a cohort of cells that expressed Foxp3 at the time of tamoxifen administration. In contrast to continuous labeling, inducible labeling of Foxp3-expressing cells with YFP avoids the constant incorporation into the labeled population of cells that transiently up-regulate Foxp3 and affords assessment of bona fide T_{reg} cell maintenance.

Foxp3$^{eGFP\text{-}Cre\text{-}ERT2}$ × R26Y mice had normal numbers of CD4 and CD8 double- and single-positive thymocytes and $CD4^+$ and $CD8^+$ T cells

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in the secondary lymphoid organs (fig. S2). Foxp3 and the associated eGFP reporter were expressed in a manner indistinguishable from expression of the Foxp3 protein in control wild-type mice, and eGFP expression was limited to Foxp3$^+$ cells (figs. S2 and S3A). YFP was not detected in eGFP$^+$ cells in the absence of tamoxifen and was restricted to Foxp3$^+$ cells upon tamoxifen administration (fig. S3, B and C). Treatment with tamoxifen resulted in three main populations of CD4$^+$ T cells: eGFP$^-$YFP–Foxp3$^-$ "non-T$_{reg}$" cells, eGFP$^+$YFP$^-$ unlabeled Foxp3$^+$ T$_{reg}$ cells, and tagged eGFP$^+$YFP$^+$ Foxp3$^+$ cells (fig. S4). We also observed a minor population of eGFP$^-$YFP$^+$ cells (<5% of YFP$^+$ cells), which may represent cells that were transiently expressing Foxp3 at the time of tamoxifen exposure. Both YFP-tagged and -untagged eGFP$^+$ T$_{reg}$ cells expressed identical amounts of Foxp3 and all other T$_{reg}$ cell phenotypic and activation markers tested thus far (figs. S4 and S5).

To evaluate long-term stability of Foxp3$^+$ T$_{reg}$ cells under basal conditions, *Foxp3$^{eGFP\text{-}Cre\text{-}ERT2}$* × R26Y mice were treated with tamoxifen and analyzed 5 to 8 months later for Foxp3 expression in YFP$^+$CD4$^+$ T cells. The overwhelming majority of YFP$^+$CD4$^+$ T cells remained eGFP-positive in the lymph nodes, spleen, Peyer's patches, skin, and lung up to 5 months after tamoxifen treatment (Fig. 1, A and B). This result was confirmed by means of intracellular staining for Foxp3 of fluorescence-activated cell sorter (FACS)–purified YFP$^+$ cells (fig. S6). Furthermore, expression of T$_{reg}$ cell–associated molecules was comparable between YFP$^+$ and YFP$^+$GFP$^+$ cells (Fig. 1C and fig. S6), and production of proinflammatory cytokines was comparably low in both subsets (Fig. 1D). The minor population of eGFP$^-$YFP$^+$ cells observed 14 days after labeling failed to expand and in fact further decreased over time (Fig. 1, C and D). Thus, we did not observe a noticeable "conversion" of T$_{reg}$ cells and their progeny into follicular T helper (TFH) or effector T cells or their dedifferentiation into naïve T cells under physiologic conditions.

Surprisingly, in euthymic mice the proportion of YFP$^+$ T$_{reg}$ cells among all Foxp3$^+$ cells at 5 to 8 months after labeling was comparable with that observed at 14 days despite continuous, albeit declining with age, output of thymus-derived T$_{reg}$ cells (Fig. 1E). Furthermore, thymectomy failed to influence the proportion of YFP$^+$ T$_{reg}$ cells among Foxp3$^+$ cells at these later time points (Fig. 1E), suggesting that the peripheral T$_{reg}$ cell pool, which is characterized by a high proportion of cycling cells (fig. S7), is not only highly stable in adult mice but is largely self-sustained with a relatively minor contribution by newly generated thymic emigrants.

We next asked whether Foxp3 can be lost upon growth factor deprivation as a validation of our T$_{reg}$ cell–fate tracking approach. Foxp3 induction and T$_{reg}$ cell homeostasis are dependent upon interleukin-2 (IL-2) receptor signaling (*14*–*19*), and antibody-mediated blockade of IL-2 or IL-2R causes contraction of the T$_{reg}$ cell population (*16*). Thus, we sought to explore whether IL-2 deprivation of mature T$_{reg}$ cells is associated with death or loss of Foxp3 expression and concomitant acquisition of immune effector cytokine production. Tamoxifen-treated *Foxp3$^{eGFP\text{-}Cre\text{-}ERT2}$* × R26Y mice were treated

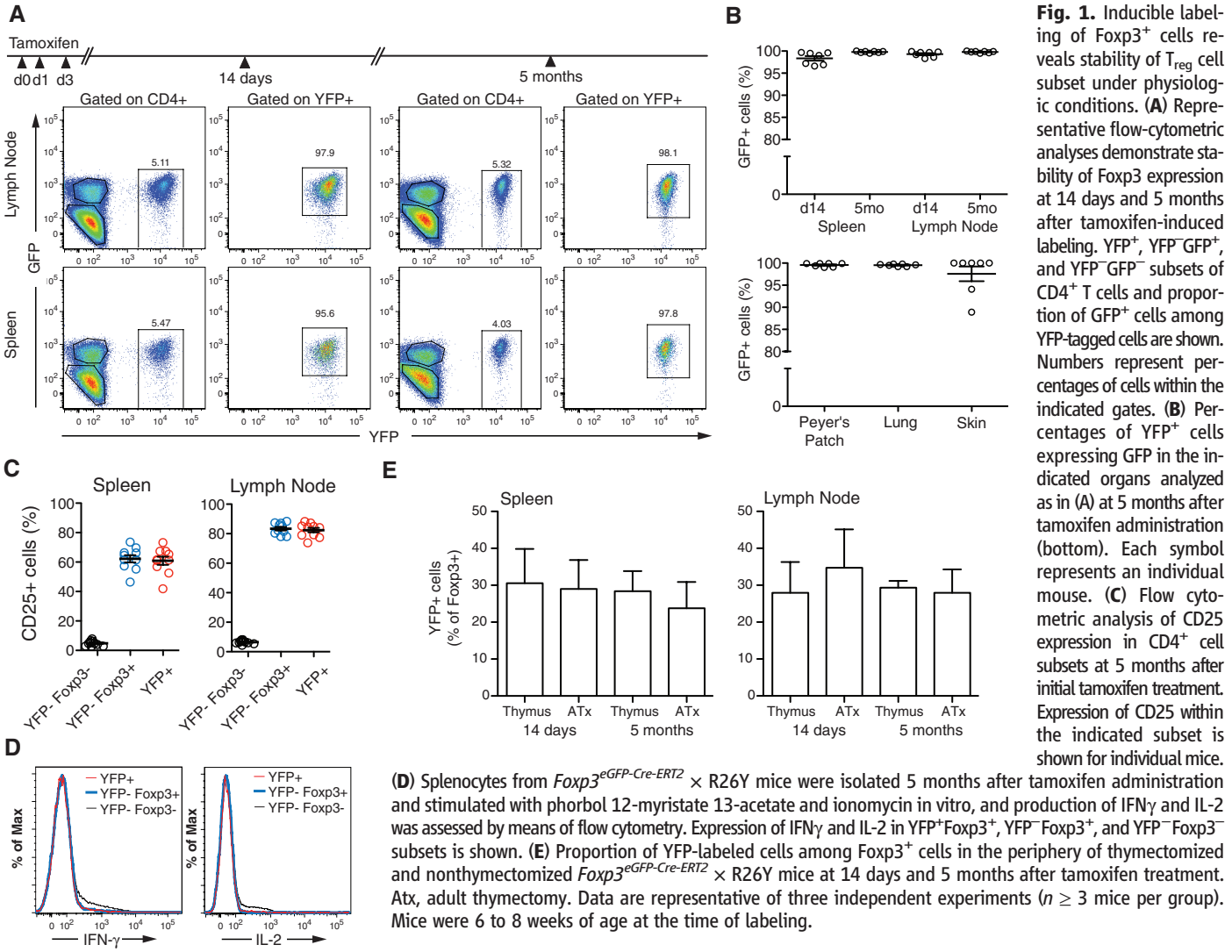


Fig. 1. Inducible labeling of Foxp3$^+$ cells reveals stability of T$_{reg}$ cell subset under physiologic conditions. (**A**) Representative flow-cytometric analyses demonstrate stability of Foxp3 expression at 14 days and 5 months after tamoxifen-induced labeling. YFP$^+$, YFP$^-$GFP$^+$, and YFP$^-$GFP$^-$ subsets of CD4$^+$ T cells and proportion of GFP$^+$ cells among YFP-tagged cells are shown. Numbers represent percentages of cells within the indicated gates. (**B**) Percentages of YFP$^+$ cells expressing GFP in the indicated organs analyzed as in (A) at 5 months after tamoxifen administration (bottom). Each symbol represents an individual mouse. (**C**) Flow cytometric analysis of CD25 expression in CD4$^+$ cell subsets at 5 months after initial tamoxifen treatment. Expression of CD25 within the indicated subset is shown for individual mice. (**D**) Splenocytes from *Foxp3$^{eGFP\text{-}Cre\text{-}ERT2}$* × R26Y mice were isolated 5 months after tamoxifen administration and stimulated with phorbol 12-myristate 13-acetate and ionomycin in vitro, and production of IFNγ and IL-2 was assessed by means of flow cytometry. Expression of IFNγ and IL-2 in YFP$^+$Foxp3$^+$, YFP$^-$Foxp3$^+$, and YFP$^-$Foxp3$^-$ subsets is shown. (**E**) Proportion of YFP-labeled cells among Foxp3$^+$ cells in the periphery of thymectomized and nonthymectomized *Foxp3$^{eGFP\text{-}Cre\text{-}ERT2}$* × R26Y mice at 14 days and 5 months after tamoxifen treatment. Atx, adult thymectomy. Data are representative of three independent experiments ($n \geq 3$ mice per group). Mice were 6 to 8 weeks of age at the time of labeling.

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with IL-2–neutralizing antibody. IL-2 deprivation resulted in a ~30% decrease in total Foxp3$^+$ T_{reg} cell numbers and a modest decrease in Foxp3 protein amounts at the single-cell level (Fig. 2, A to C). Moreover, a small subset of YFP$^+$ cells was completely devoid of Foxp3. We did not, however, observe derepression of proinflammatory cytokines [interferon-γ (IFNγ), IL-17, IL-2, and tumor necrosis factor–α (TNFα)] by YFP$^+$, YFP$^-$GFP$^+$ T_{reg} cells with decreased Foxp3 levels, or eGFP$^-$YFP$^+$ cells which had lost Foxp3 expression (fig. S8). These results demonstrate that upon IL-2 deprivation, the bulk of T_{reg} cells maintained Foxp3 expression albeit at a moderately decreased level, yet sufficient to prevent acquisition of an effector cell phenotype. Nevertheless, it is possible that IL-2 deprivation under conditions of overwhelming infection might result in a loss of Foxp3 in some T_{reg} cells (*20*).

Lymphopenia may also facilitate loss of Foxp3 expression in expanding T_{reg} cells. Despite use of purified T_{reg} cells in previously published transfer studies, the possibility of outgrowth of a small number of contaminating non-T_{reg} cells and stress associated with cell transfer remain potential caveats in interpretation of these results. To circumvent these issues, we used sublethal irradiation to evaluate stability of Foxp3 expression under lymphopenic conditions. Tamoxifen-treated *Foxp3*$^{eGFP\text{-}Cre\text{-}ERT2}$ × R26Y mice were irradiated (6 Gy), and the proportion of eGFP$^+$ and Foxp3$^+$ cells among YFP$^+$CD4$^+$ T cells was evaluated 14 days after irradiation. De-

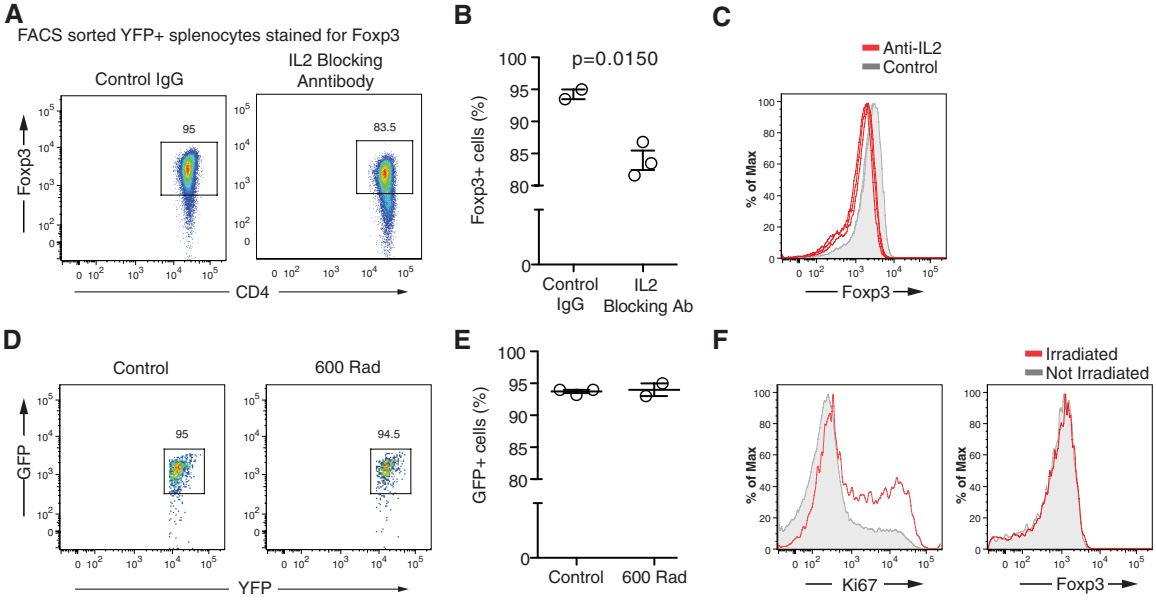


Fig. 2. IL-2 deprivation results in a small but detectable decrease in Foxp3 expression. (**A** to **C**) Tamoxifen-treated *Foxp3*$^{eGFP\text{-}Cre\text{-}ERT2}$ × R26Y mice were injected with IL-2–blocking antibody or control immunoglobulin G (IgG), and Foxp3 expression in FACS-sorted YFP$^+$ cells was assessed 9 days later. (A) Intracellular Foxp3 staining of splenic YFP$^+$ cells. Numbers represent percentages of cells in the indicated gate. (B) Percentages of YFP$^+$ cells expressing Foxp3. Each symbol represents an individual mouse. (C) Relative expression of Foxp3 in YFP$^+$ cells from spleens of mice treated with IL-2–blocking antibody. Data are representative of three independent experiments ($n \geq 2$ mice per group). Mice were 6 to 8 weeks of age at the time of antibody administration. (**D** to **F**) T_{reg} cells maintain stable expression of Foxp3 under lymphopenic conditions. Lymphopenia was induced in tamoxifen-treated *Foxp3*$^{eGFP\text{-}Cre\text{-}ERT2}$ × R26Y mice upon irradiation (6 Gy), and mice were analyzed 14 days later. (D) Representative flow-cytometric analysis of Foxp3 expression among cells isolated from spleens of control and irradiated mice 14 days after irradiation. Numbers represent percentages of cells in the indicated gate. (E) Percentages of GFP$^+$ cells among YFP$^+$ cells in spleens 14 days after irradiation. Each symbol represents an individual mouse. (F) Representative flow-cytometric analysis of Foxp3 and Ki67 expression by YFP$^+$ splenocytes on day 14 after irradiation. Similar data were obtained from cells isolated from lymph nodes of irradiated mice. Data are representative of ≥2 independent experiments, each with ≥2 mice per group. Mice were 8 to 12 weeks of age at the time of irradiation.

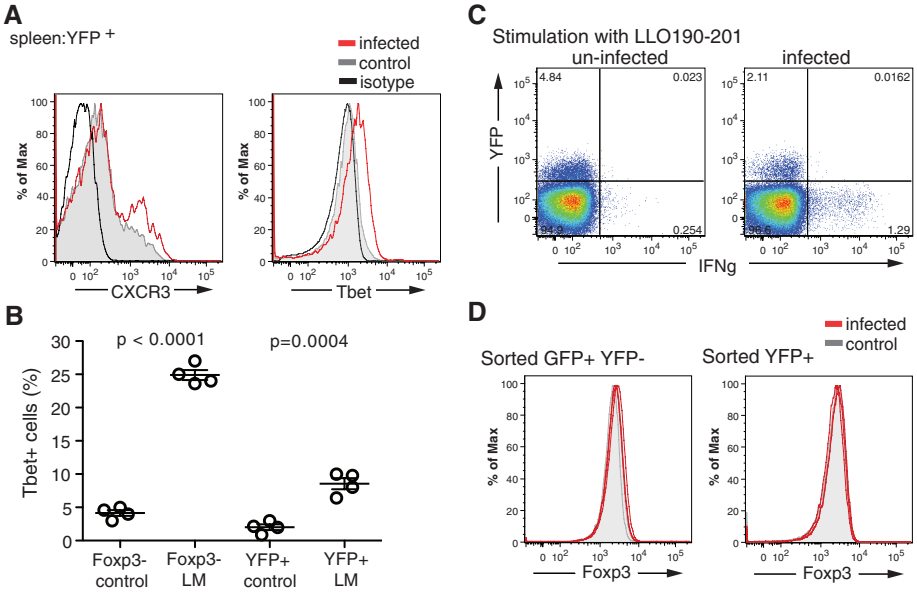


Fig. 3. Th1-type inflammation does not induce loss of Foxp3. Tamoxifen-treated *Foxp3*$^{eGFP\text{-}Cre\text{-}ERT2}$ × R26Y mice were infected intravenously with 5 × 10^3 colony-forming units (CFU) of *Listeria monocytogenes* and analyzed 9 days later. (**A**) Flow-cytometric analysis demonstrates up-regulation of T-bet and CXCR3 by splenic YFP$^+$CD4$^+$ cells in infected mice. (**B**) Percent of T-bet positive cells among Foxp3$^-$ and YFP$^+$ splenocytes as determined in (A). Each symbol represents an individual mouse. (**C**) Antigen-specific production of IFNγ in splenocytes restimulated with LLO190-201 peptide. CD4$^+$ T cell gate is shown; numbers represent percentages of cells in corresponding quadrants. (**D**) Foxp3 expression in sorted (top) YFP$^-$GFP$^+$ or (bottom) YFP$^+$ cells from *L. monocytogenes* infected or control mice 9 days after infection. Data are representative of three independent experiments with ≥2 mice per group each. Mice were 6 to 8 weeks of age at the time of infection.

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spite a marked increase in numbers of dividing T_{reg} cells reflected in heightened expression of Ki67 in YFP^+ and YFP^-T_{reg} cells, GFP and Foxp3 expression was preserved in YFP^+ cells (Fig. 2, D to F). We considered that perturbation of the 3′UTR in *Foxp3*$^{eGFP\text{-}Cre\text{-}ERT2}$ × R26Y mice may have resulted in increased Foxp3 mRNA or protein stability. However, Foxp3 mRNA decay in the presence of actinomycin D in T_{reg} cells from *Foxp3*$^{eGFP\text{-}Cre\text{-}ERT2}$ × R26Y mice was more pronounced than that in T_{reg} cells from the previously described *Foxp3*^{GFP} mice, in which GFP sequence was inserted into the 5′ end of the Foxp3 coding sequence, leaving the 3′UTR intact (fig. S9) (*4*). To compare the stability of Foxp3 protein expression in T_{reg} cells from *Foxp3*$^{eGFP\text{-}Cre\text{-}ERT2}$ × R26Y mice with that in *Foxp3*^{GFP} mice in vivo, $CD4^+GFP^+$ cells were doubly purified by means of FACS from each mouse strain and cotransferred with $Ly5.1^+CD4^+$ T cells into TCRβδ-deficient mice. Three weeks after transfer, the majority (~90%) of $CD4^+Ly5.2^+$ cells maintained GFP and Foxp3 protein expression, and their percentages among cells transferred from both mouse strains were similar (fig. S10), further supporting the stability of the T_{reg} cell lineage and indicating that the modification of the 3′UTR in *Foxp3*$^{eGFP\text{-}Cre\text{-}ERT2}$ × R26Y mice cannot account for this observation.

Previous studies suggested that during infection, exposure of T_{reg} cells to pro-inflammatory cytokines results in a loss of Foxp3 expression and T_{reg} cell conversion into effector T cells and was interpreted as a means to facilitate appropriate immune responsiveness (*21*). To examine the fate of $Foxp3^+$ T_{reg} cells during infection, *Foxp3*$^{eGFP\text{-}Cre\text{-}ERT2}$ × R26Y mice were treated with tamoxifen, infected with a sublethal dose of *Listeria monocytogenes*, and analyzed for T_{reg} cell stability at the peak of the immune response. After infection, we observed considerable up-regulation of the T helper 1 transcription factor T-bet and CXCR3 in $Foxp3^-$, $Foxp3^+$, and YFP^+ $CD4^+$ cells (Fig. 3, A and B, and fig. S11) and IFNγ production by effector $CD4^+$ T cells in response to listeriolysin O (LLO) peptide (Fig. 3C and fig. S11). Nevertheless, essentially all YFP^+ T_{reg} cells maintained expression of Foxp3 (Fig. 3D), including those with increased T-bet expression. Foxp3 stability was also observed in $T\text{-}bet^+Foxp3^+$ cells generated upon induction of a strong IFNγ-dominated Th1 inflammatory response upon antibody-mediated cross-linking of CD40 (fig. S12) (*22*). Cross-linking of CD40 after tamoxifen-induced labeling resulted in a marked up-regulation of T-bet and CXCR3 in $Foxp3^+$ and $Foxp3^-$ cells (fig. S12). YFP-tagged cells demonstrated increased expression of CXCR3 and T-bet but at best marginally increased IFNγ and IL-2 production (fig. S12). Furthermore, T_{reg} cells maintained expression of Foxp3 at 14 days and up to 5 months after CD40 cross-linking (fig. S12). Thus, T_{reg} cells maintain heritable Foxp3 expression even after up-regulation of the Th1 transcription factor T-bet in the course of Th1-dependent antibacterial infection and CD40 cross-linking driven Th1 inflammatory response. T-bet and CXCR3 induction in T_{reg} cells was recently shown to facilitate their ability to restrain Th1 inflammation (*22*).

Lastly, to evaluate the stability of $Foxp3^+$ cells in an autoimmune inflammatory setting, highly purified pancreatic-islet-antigen–specific GFP^+ T_{reg} cells from NOD.*Foxp3*^{gfp}.BDC2.5 TCR-transgenic mice (*23*) were transferred into prediabetic, lymphoreplete NOD.Thy1.1 recipients at 12 weeks of age, a time point at which pancreatic infiltration is well established. Four weeks later, donor cells were enriched in the inflamed pancreas but retained expression of Foxp3 and, in contrast to recipient effector cells, expressed no detectable IL-17 or IFNγ (Fig. 4, A to C). In parallel experiments, T_{reg} cell stability was evaluated in the K/BxN TCR-transgenic model, in which reactivity to a ubiquitous self-antigen leads IL-17– and gut microbiota–dependent generation of arthritogenic auto-antibodies (*24*). Highly purified GFP^+ T_{reg} cells from K/BxN.*Foxp3*^{gfp} mice were transferred into 20-day-old K/BxN recipients—at the initiation of the very robust inflammatory process—and again remained almost exclusively $Foxp3^+$ and failed to produce IL-17 in lymphoid organs or the lamina propria (Fig. 4, D to F). Thus, T_{reg} cells maintained stable Foxp3 expression and showed no evidence of effector cytokine production under conditions of autoimmune inflammation.

In contrast to our findings, a recent study reported a marked loss of Foxp3 expression in YFP-tagged $Foxp3^+$ T_{reg} cells in R26Y × Foxp3-Cre BAC transgenic mice and acquisition of effector function by YFP^+Foxp3^- T cells (*25*). Likewise, upon transfer into lymphopenic recipients $Foxp3^+$ T_{reg} cells were found to lose Foxp3 expression and differentiate into TFH cells in the

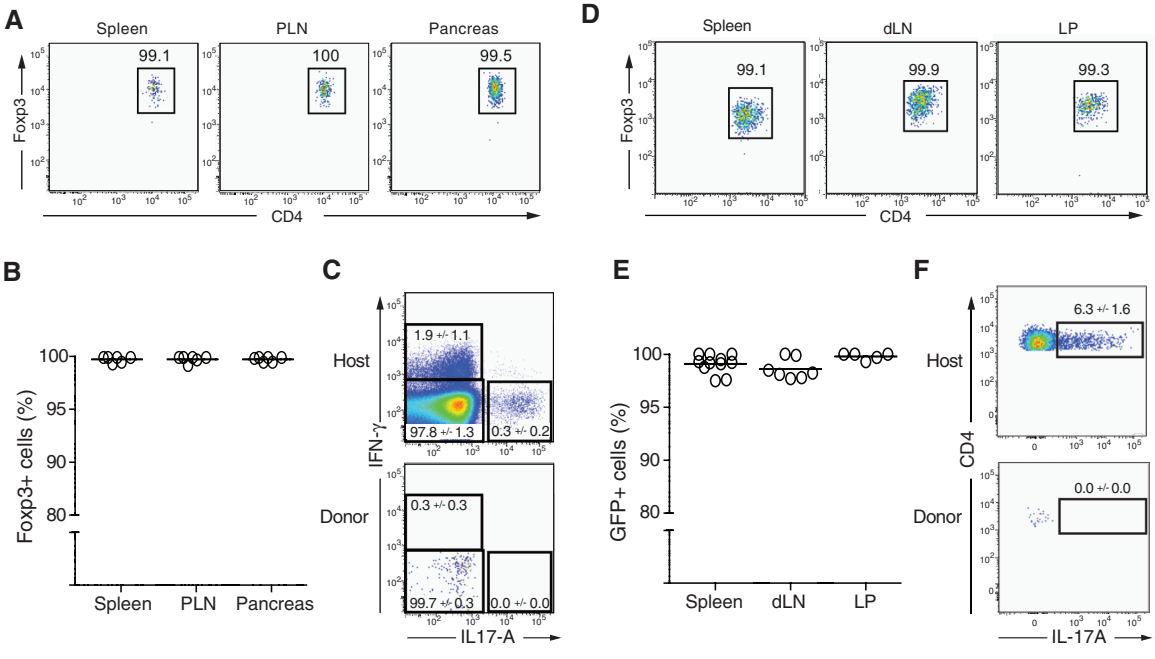


Fig. 4. Foxp3 expression is stable in T_{reg} cells during autoimmune inflammation. (**A** to **C**) Highly purified GFP^+ cells from BDC2.5.*Foxp3*^{eGFP} mice were transferred into 12-week-old prediabetic NOD.Thy1.1 recipients, and the spleen, pancreatic lymph nodes (PLN), or pancreatic infiltrates were analyzed 4 weeks later. (A) Representative Foxp3 staining of $Thy1.2^+CD4^+$ donor cells. (B) Tabulation of individual mice in three independent experiments. (C) Intracellular IFNγ and IL-17 staining of host and donor $CD4^+$ cells (numbers represent percentage of cells in the gate ± SD; $n = 3$ to 6 mice per group). (**D** to **F**) Highly purified $CD4^+GFP^+$ cells from 20-day-old K/BxN.*Foxp3*^{gfp} mice were transferred into age-matched K/BxN.CD45.2 mice. (D) Spleen, joint-draining lymph node, and small intestine lamina propria (LP) were collected from arthritic 35-day-old recipients and $CD45.1^+CD4^+$ donor cells analyzed for Foxp3-GFP expression. (E) Tabulation of individual mice in two independent experiments. (F) Expression of IL-17a by host and donor $CD4^+$ cells from the lamina propria (numbers represent percentage of cells in the gate ± SD; $n = 3$ mice per group). Data are representative of two independent experiments.

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Peyer's patches (*26*). Seemingly unstable Foxp3 expression observed in these and other studies (*21*) can be due to cellular stress, the presence of few contaminating Foxp3$^-$ cells or cells which underwent transient Foxp3 up-regulation, or recently generated Foxp3$^+$ cells on their way to stable Foxp3 expression yet still able to expand and differentiate into effector T cells in lymphopenic or nonlymphopenic settings. Consistent with the latter notion, loss of Foxp3 expression by a minor population of YFP$^+$ cells was observed during induction of Foxp3 in peripheral YFP$^-$GFP$^-$CD4$^+$ T cells under lymphopenic conditions. In contrast, YFP$^+$GFP$^+$ T_{reg} cells maintained Foxp3 expression in these experiments (fig. S13). Furthermore, transient expression of Foxp3 during retinoic acid receptor (RAR)–related orphan receptor–γ (RORγ)–dependent differentiation of Th17 cells was visualized through activation of the R26Y allele by Cre recombinase constitutively expressed under control of the endogenous *Foxp3* locus (*27*). Additionally, differences in regulation of expression of *Foxp3* bacterial artificial chromosome (BAC) transgene and the endogenous *Foxp3* locus encoding Cre can also affect the discrepant outcomes of YFP-tagging of T_{reg} cells in the corresponding experimental models.

We demonstrated that the T_{reg} cell lineage is remarkably stable under physiologic conditions and after a variety of challenges. Stable Foxp3 expression in committed T_{reg} cells is probably facilitated by a positive autoregulatory loop (*28*). Our results also suggest that continuous self-renewal of the established T_{reg} cell population combined with heritable maintenance of Foxp3 expression serves as a major mechanism of maintenance of this lineage in adult mice.

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Dendritic Discrimination of Temporal Input Sequences in Cortical Neurons

Tiago Branco, Beverley A. Clark, Michael Häusser*

The detection and discrimination of temporal sequences is fundamental to brain function and underlies perception, cognition, and motor output. By applying patterned, two-photon glutamate uncaging, we found that single dendrites of cortical pyramidal neurons exhibit sensitivity to the sequence of synaptic activation. This sensitivity is encoded by both local dendritic calcium signals and somatic depolarization, leading to sequence-selective spike output. The mechanism involves dendritic impedance gradients and nonlinear synaptic *N*-methyl-D-aspartate receptor activation and is generalizable to dendrites in different neuronal types. This enables discrimination of patterns delivered to a single dendrite, as well as patterns distributed randomly across the dendritic tree. Pyramidal cell dendrites can thus act as processing compartments for the detection of synaptic sequences, thereby implementing a fundamental cortical computation.

In sensory pathways, the relative timing of spikes from different neuronal populations can represent features of stimuli (*1*–*4*). A fundamental problem in cortical sensory processing is, thus, the discrimination of different spatiotemporal sequences of inputs (*5*). Although networks composed of simple neurons can, in principle, decode temporal sequences, the size and complexity of such networks can be greatly reduced if individual neurons can perform temporal decoding (*6*, *7*). Dendritic trees might contribute to this decoding, because they are highly nonlinear devices that can locally process and integrate synaptic signals (*8*–*10*). For example, spatiotemporally clustered inputs trigger dendritic spikes (*11*–*15*), which can generate independent functional subunits, enhancing the computational potential of the neuron (*16*–*18*) and encoding spatial and temporal input synchrony. Whether these nonlinear dendritic properties can be exploited to perform higher-order computations such as temporal sequence detection is unknown. In 1964, Wilfrid Rall predicted that, because dendrites act as a delay line, activation of synapses along a dendrite in different directions should produce different responses at the soma (*19*). Although the dendrites of retinal neurons exhibit such direction selectivity (*20*–*22*), experimental investigation of the sensitivity to spatiotemporal sequences of synaptic activation in cortical pyramidal cell dendrites has been a challenge due to the difficulty in delivering the spatiotemporal input patterns with the necessary submillisecond and submicron precision.

To test the sensitivity of single dendrites to the order of activation of a defined set of synapses, we controlled spatiotemporal input patterns using multisite, two-photon glutamate uncaging at identified dendritic spines (*15*, *23*) in layer 2/3 pyramidal neurons of somatosensory and visual cortex. We first studied the sensitivity of single dendrites to an ordered sequence of synaptic activation in opposite directions, selecting 8 to 10 spines on single basal and apical oblique dendritic branches (Fig. 1, A and B). Activating each site in isolation (Fig. 1C) produced synaptic responses [glutamate excitatory postsynaptic potentials (gluEPSPs)] within physiological parameters (fig. S1). Sequential activation of spines from the dendritic branch to the soma (IN) or from the soma to the tip (OUT) produced strongly directionally sensitive responses. The IN direction always produced a larger somatic response than the OUT direction (Fig. 1, D and E) (31 ± 4% increase, $P < 0.0001$,

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n = 20 observations, corresponding to a mean peak voltage difference of 2.8 ± 0.4 mV), even when stimulating only three inputs (fig. S2). The EPSP peak was dependent on input velocity (Fig. 1, F and G), with optimal direction sensitivity at 2.6 ± 0.5 μm/ms (Fig. 1H). Axonal action potential output was highly directionally sensitive, with a higher spike probability in the IN direction than the OUT direction (38 ± 9% increase, P = 0.0013, n = 7) (Fig. 1, I to K).

The readout of sequence sensitivity was not just electrical but also chemical, as local dendritic Ca^{++} signals also showed strong direction and velocity sensitivity (Fig. 2, A to C). Peak local dendritic Ca^{++} signals were always larger in the IN versus the OUT direction (48 ± 13% increase, P = 0.0047, n = 6) and varied significantly with input velocity [P = 0.0431 and 0.0194 for IN and OUT, respectively; analysis of variance (ANOVA) test], with the optimal velocity for direction sensitivity being similar to that of the somatic EPSP (2.0 ± 0.4 μm/μs) (Fig. 2, D and E). The spatial Ca^{++} profiles along the dendrite were also different for the IN and OUT directions (Fig. 2, C and F).

What are the mechanisms underlying direction and velocity sensitivity in single dendrites? First, the somatic voltage responses to input sequences were markedly supralinear (Fig. 3A) (peak voltage = 223 ± 9% of the arithmetic sum, P < 0.0001) and voltage-dependent (fig. S3, B to D) and did not exhibit a clear somatic threshold or a step increase in dV/dt (where V is voltage, and t is time) (fig. S3A) (*13*, *15*), but rather developed gradually with increasing numbers of activated synapses (Fig. 3B). Next, we investigated the role of *N*-methyl-D-aspartate (NMDA) receptors (NMDARs), given their important contribution to dendritic nonlinearities in pyramidal cells (*13*, *16*–*18*, *24*). The NMDAR blocker D-AP5 abolished EPSP supralinearity (103 ± 3% of linear sum, P = 0.336, n = 8) (Fig.

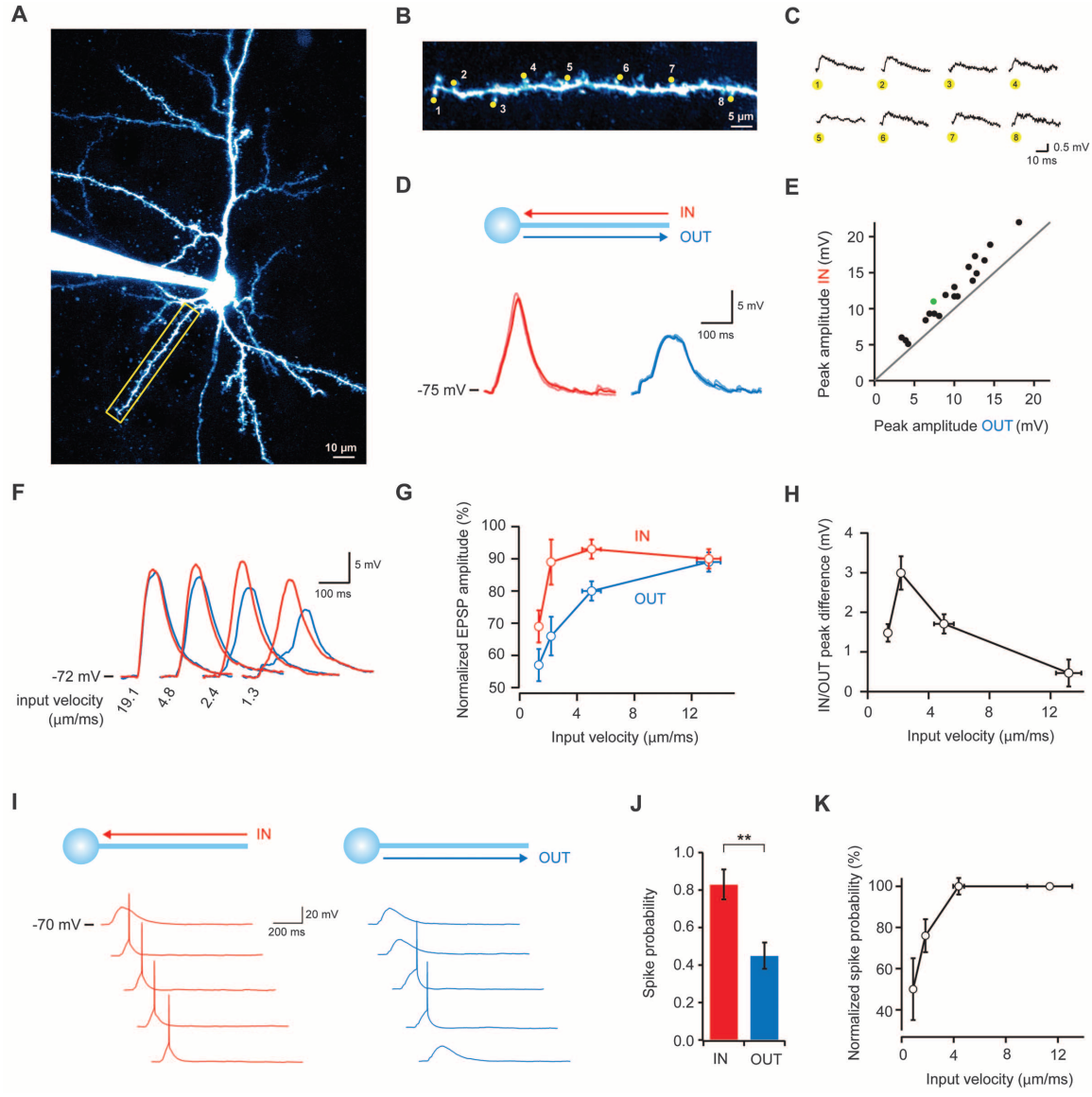


Fig. 1. Single dendrites are sensitive to the direction and velocity of synaptic input patterns. (**A**) Layer 2/3 pyramidal cell filled with Alexa 594 dye; yellow box indicates the selected dendrite. (**B**) Uncaging spots (yellow) along the selected dendrite. (**C**) Average individual uncaging responses at the soma. (**D**) Somatic responses to IN (red) and OUT (blue) directions at 2.3 μm/ms (averages denoted by bold lines). (**E**) Plot comparing peak amplitudes for IN and OUT sequences at the optimal velocity for direction selectivity [green circle, example shown in (D)]. (**F**) Direction-selective responses at different velocities. (**G**) Relation between peak voltage and input velocity (values normalized to the maximum response in the IN direction for each cell, n = 15). Error bars indicate SEM. (**H**) Relation between direction selectivity and input velocity (n = 15). (**I**) Direction selectivity of spike probability; population data shown in (**J**) (**P = 0.0013, n = 7). (**K**) Relation between spike probability and velocity (n = 7, average of both directions).

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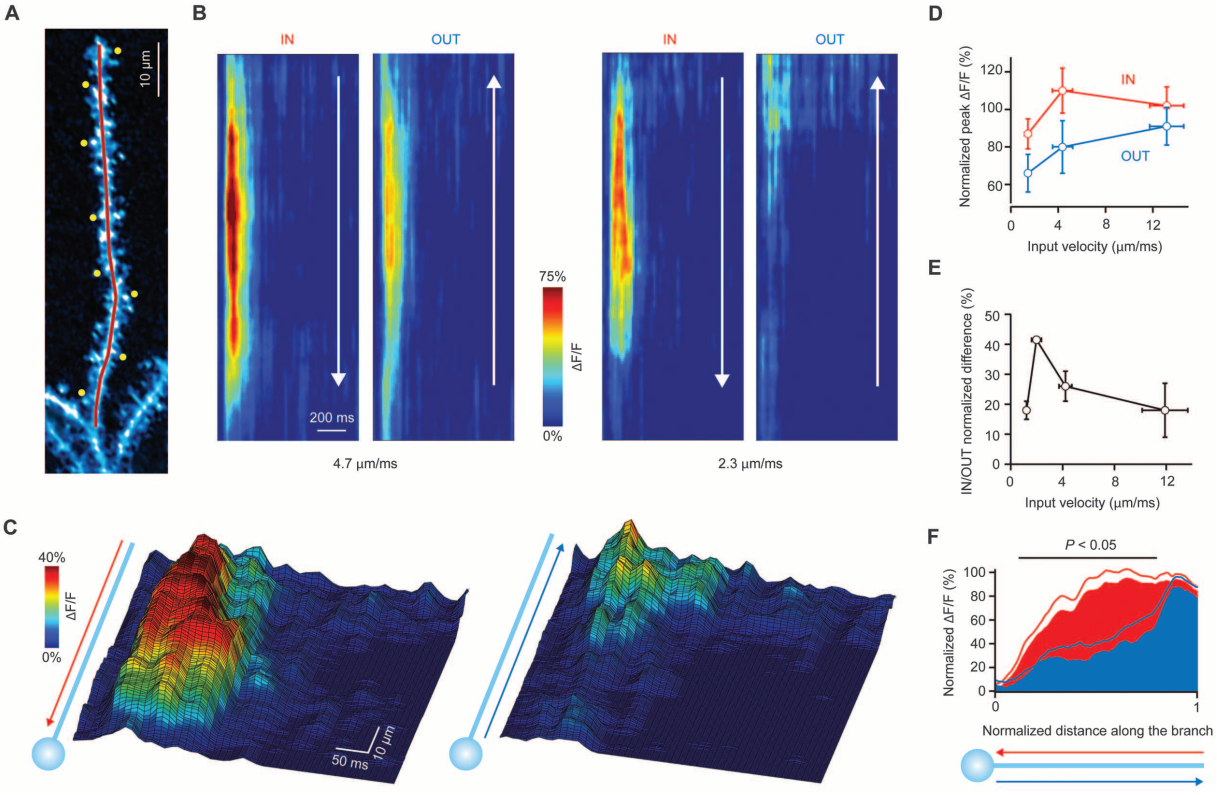


Fig. 2. Dendritic calcium influx is direction and velocity sensitive. (**A**) Basal dendrite of a layer 2/3 pyramidal neuron. Uncaging locations indicated in yellow; linescan profile used for Ca^{++} imaging in red. (**B**) Spatiotemporal profile of Ca^{++} signals triggered by IN and OUT input patterns at two different input velocities. (**C**) Three-dimensional plot of the data in (B) (2.3 μm/ms). (**D**) Relation between Ca^{++} signals and input velocity ($\Delta F/F$ values, where F is fluorescence, normalized to the mean $\Delta F/F$ of all velocities in the IN direction of each cell). Error bars indicate SEM. (**E**) Relation between direction selectivity of Ca^{++} signals and input velocity. (**F**) Average spatial profile of the integrated Ca^{++} transient across the dendrite (n = 5 cells) (lines indicate SEM; bar indicates region of statistical significance).

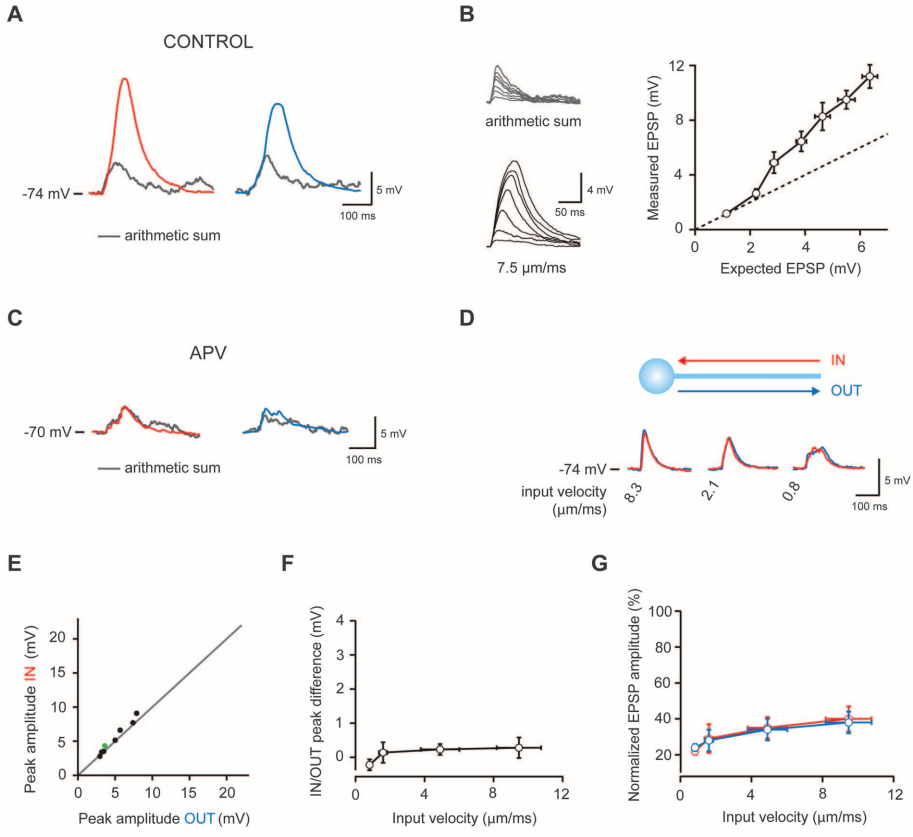


Fig. 3. NMDA receptor activation is required for robust velocity and direction coding. (**A**) Somatic responses to IN and OUT sequences (red and blue traces, respectively) and linear sum of the individual synaptic responses (gray traces). (**B**) (Left) Activating more synapses (1 to 7) produces EPSPs that are progressively larger than the linear sum of the individual responses. (Right) Summary plot of eight cells. Solid line, experimental data points; dashed line, unity line. Error bars indicate SEM. (**C**) Same as in (A), but in the presence of AP5, which blocks the supralinearity. (**D**) Velocity and direction sensitivity is abolished in AP5. (**E**) Comparing peak amplitudes for IN and OUT sequences at the optimal velocity for direction selectivity [green circle, example shown in (C)] (compare with Fig. 1E). (**F**) Summary plot of direction selectivity versus velocity (compare with Fig. 1G). (**G**) Reduced velocity sensitivity in AP5 (EPSPs normalized to the average IN maximum in control cells in the absence of D-(–)-2-amino-5-phosphonopentanoic acid) (compare with Fig. 1H).

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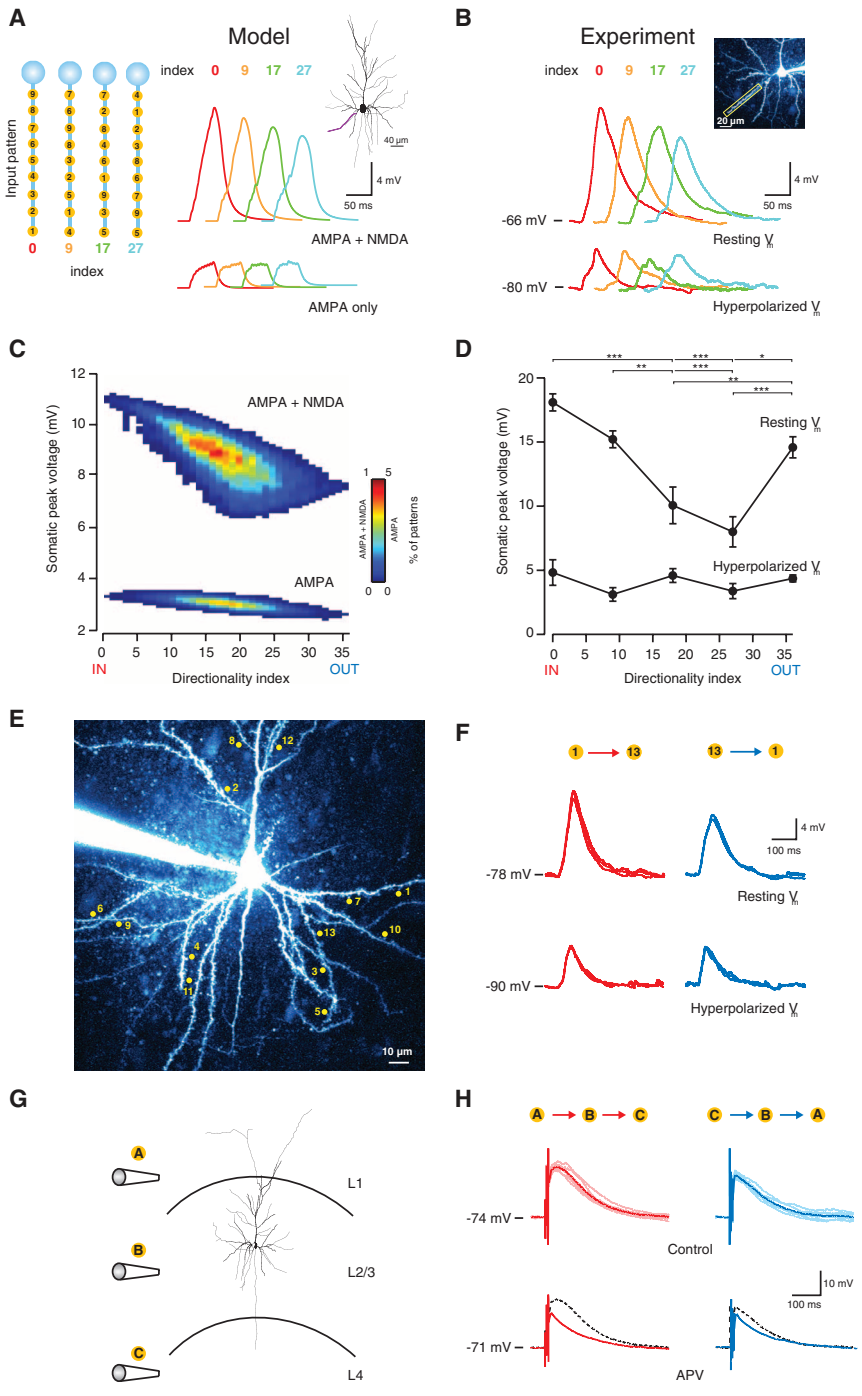


Fig. 4. Dendritic discrimination of complex input sequences. (**A**) Random patterns of nine inputs were generated and sorted by their directionality index (*35*). Four sample patterns (numbers showing activation order) and corresponding directionality index are shown. Patterns were played into a single dendrite in a pyramidal cell model or (**B**) experimentally in single pyramidal cell dendrites. The peak response is different for different patterns, and responses become similar with hyperpolarization (or without NMDA receptors in the model). (Inset) Dye-filled neuron with dendrite used in the experiment enclosed by a yellow box. Vm, membrane potential. (**C**) Pattern separability (measured by somatic peak EPSP distribution) was much greater with NMDA conductances present. (**D**) Peak EPSP voltage measured experimentally depends on input-pattern similarly to the model (C). Asterisks indicate significant differences between EPSP peaks for the example shown; hyperpolarization reduces the ability to discriminate different patterns. Error bars indicate SEM. (**E**) A layer 2/3 pyramidal cell with uncaging spots (yellow) randomly distributed across eight dendrites. (**F**) Somatic responses to sequential uncaging in forward (red traces) or reverse sequence (blue traces; averages denoted by bold lines) are different at resting potential and become similar upon hyperpolarization. (**G**) Three stimulation electrodes (denoted by letters A, B, and C) were placed in different cortical layers and activated in two different sequences. (**H**) The EPSP depends on the sequence and on NMDAR activation. Black dashed lines denote control response.

3C), consistent with amplification via NMDA-dependent regenerative signal boosting and NMDA spikes (*25*). The NMDAR blocker also abolished direction sensitivity (Fig. 3, D to F) (IN response 8 ± 3% larger than the OUT response; peak voltage difference of 0.4 ± 0.4 mV, significantly smaller than control, $P = 0.0011$) and velocity sensitivity (Fig. 3G), as well as any detectable dendritic calcium signals (fig. S4).

To probe the biophysical basis of direction sensitivity, we used a detailed compartmental model of layer 2/3 pyramidal cells. Direction sensitivity could be reproduced with a relatively simple model, with passive dendrites and synapses containing AMPA and NMDA conductances (fig. S5). The simulations showed that the mechanism of direction sensitivity in single dendrites results from the interaction of two factors: (i) the gradient of input impedance along the dendrite combined with (ii) the highly nonlinear voltage dependence of the NMDAR conductance. This leads to asymmetric recruitment of NMDA receptors when activating synapses in different sequences, such that sequences starting from regions of high input impedance (the tip of the dendrite) generate more initial local depolarization and, hence, more cumulative NMDAR activation. We verified the differential recruitment of nonlinearities between proximal and distal synapses with calcium imaging and glutamate uncaging in different regions of the same dendrite (fig. S6). The relative simplicity of this mechanism suggests that it should be general; indeed, the vast majority of dendrites across a wide range of morphologies exhibit sufficient impedance gradients to permit substantial direction sensitivity (fig. S7). We confirmed this experimentally by showing that neurons with very different morphologies—layer 5 pyramidal cells and hippocampal dentate gyrus granule cells—also exhibit robust direction sensitivity (fig. S8). This is especially relevant in the latter, as they receive layered input from the entorhinal cortex, with the lateral cortex projecting distally and the medial cortex proximally.

Can this mechanism be used to discriminate more complex patterns of input? We first assessed discrimination of random patterns by a single dendrite (Fig. 4, A and B). In both the model (Fig. 4, A and C) and the experiments (Fig. 4, B and D), different sequences of the same inputs produced a wide range of somatic EPSP amplitudes ($P < 0.05$, ANOVA on each cell; mean difference between experimentally tested patterns = 2.3 mV ± 0.22 mV; 39 ± 8% of all possible sequence comparisons were significant, $P < 0.05$, t test with Bonferroni correction, $n = 7$), which was dependent on NMDA receptor activation (mean difference between patterns = 0.69 ± 0.13 mV, $P < 0.0001$) (Fig. 4, C and D). This is because synapses at different locations along the dendrite differentially influence each other, depending on their relative timing. The broad EPSP amplitude distribution reflects the discriminability of different sequences, with the likelihood of discriminating any two sequences (>1-mV

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peak difference) being 40%. This distribution was insensitive to feedback inhibition but shifted to a smaller mean with the same variance with feedforward inhibition, suggesting that the same NMDA-dependent mechanism can represent the combined spatiotemporal sequence of excitation and inhibition (fig. S9). Whereas synaptic interactions are maximal if synapses are on the same dendrite, voltage can spread to other parts of the dendritic tree, the extent depending on the tree geometry and biophysical properties. We therefore examined whether neurons could also discriminate patterns of input delivered to multiple dendrites. By activating multiple spines across several dendrites, it was possible to discriminate the response to activation of different sequences of distributed input patterns (Fig. 4, E and F) (mean EPSP peak difference = 3.3 ± 0.8 mV, P = 0.0065, n = 9; tested sequences had no net directionality; see fig. S10, E and F, and supporting online material text). This discriminability was fully reproduced by our model (fig. S10, A and B) and was severely reduced upon hyperpolarization (0.58 ± 0.2 mV, P = 0.0003) (Fig. 4F) and by block of NMDA receptors (fig. S10B). We further extended these results using the model, in which input sequences could be reliably discriminated over a wide range of spatial input distributions (fig. S10, C and D) and experimentally by showing that sequential activation of multiple input pathways could be discriminated (mean peak difference in control 2.8 ± 0.3 mV versus 0.9 ± 0.2 mV in AP5, P = 0.0121, n = 6) (Fig. 4, G and H).

We have shown that single cortical pyramidal cell dendrites can encode the temporal sequence of synaptic input. The underlying mechanism relies on the interplay between nonlinear activation of synaptic NMDA receptors and the impedance gradient along dendritic branches, two fundamental biophysical features common to most neurons in the brain. The active, regenerative nature of this mechanism contrasts with the classic, passive directional selectivity proposed by Rall (*19*), which requires electrotonically very long dendrites. Instead, the NMDA-dependent mechanism produced strong sensitivity to the direction of synaptic input, even in short pyramidal cell dendrites, making it more general and sensitive to synaptic input, robust against timing jitter, and further enhanced and tunable by depolarization [such as in network UP states (*26*, *27*)]. Different input sequences also lead to differential dendritic Ca^{++} signals, raising the possibility that they will engage plasticity mechanisms to different extents (*28*, *29*). The large dynamic range conferred by NMDA receptor activation allowed for high discriminability of multiple temporal sequences, both when inputs were on the same dendrite or when they were dispersed over the dendritic tree. This further extends the range of computational and plasticity mechanisms that have recently been described in dendrites (*10*, *13*, *15*–*18*, *30*, *31*). In particular, this sensitivity to temporal input sequences may be relevant for detecting features of sensory stimuli and for encoding the speed and directionality of waves of activity propagating in the cortex (*32*–*34*). It is also especially relevant for circuits with layered input such as the hippocampus, where this mechanism could be used by dentate gyrus granule cells to directly detect the sequence of entorhinal cortex activation. These computations are conventionally thought to be implemented at the level of neural populations, and thus, our results represent a demonstration of the power of dendrites for solving computational problems in the brain.

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35. Materials and methods are available as supporting material on *Science* Online.

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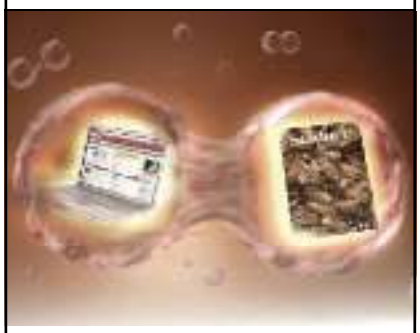
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The Division of Endocrinology and Metabolism at the University of Rochester School of Medicine is seeking new research faculty members of all ranks. Candidates must have an M.D. or Ph.D. and should be performing endocrine-related basic or translational research. The Division is under new leadership, has recently moved into newly renovated space, and is seeking to rapidly expand its academic programs. Thus, the University of Rochester offers an exciting opportunity for research scientists interested in endocrinology. Please send an e-mail with curriculum vitae and cover letter to **Stephen Hammes,** chief, Division of Endocrinology and Metabolism, University of Rochester School of Medicine. **E-mail: stephen_hammes@urmc.rochester.edu.**

The University of Rochester has a strong commitment to principles of diversity and, in that spirit, actively encourages applications from groups underrepresented in higher education.

NEUROBIOLOGY FACULTY POSITION. The Department of Biology at Clark University, Worcester, Massachusetts (**website: http://www.clarku.edu/departments/biology/**), invites applications for a tenure-track appointment at the rank of **ASSISTANT PROFESSOR**, to begin fall 2011. The successful candidate will be expected to develop an externally funded research program in any area of neurobiology involving Ph.D. and undergraduate students. The candidate will teach Physiology, and courses in neurobiology and related areas of expertise. Electronic copies of curriculum vitae, research and teaching interests, three publications, and three letters of reference should be received by the Search Committee prior to November 1, 2010, for full consideration (**e-mail: neuro@clarku.edu**). Please see (**website: http://www.clarku.edu/facultyjobs/**) for full position description. Inquiries should be directed to Susan Foster (**e-mail: sfoster@clarku.edu**). *Affirmative Action/Equal Opportunity Employer. Minorities and women are strongly encouraged to apply.*

POSITIONS OPEN

SENIOR FACULTY POSITIONS in Human Immunology at the Vaccine and Gene Therapy Institute-Florida

The Vaccine and Gene Therapy Institute-Florida (VGTI-FL) is seeking outstanding senior **IMMUNOLOGISTS** to lead programs in basic and translational human immunology. VGTI-FL is one of the internationally acclaimed research institutes that have recently located to Florida. Research at the institute focuses on Global Health initiatives utilizing systems biology approaches to study the human adaptive and innate immune response to prevent and treat chronic infectious disease and cancer as well as identify immune defects of the aged to boost their immunity. VGTI-FL has ongoing basic and clinical research programs with the University of Miami, Moffitt Cancer Center, and Martin Memorial Hospital. The institute will occupy a new 100,000 square foot facility that will be located in Port St. Lucie, Florida on the Atlantic coast in a Biotech cluster that includes Torrcy Pines Institute for Molecular Studies, the Mann Research Center, and Martin Memorial Hospital as well as the Max Planck Institute and the Scripps Research Institute Florida within 30 minutes drive. Successful candidates (M.D. or Ph.D.) will have an established extramurally funded research program and a strong publication record in one of the areas of immunology described above. The positions have highly competitive salaries and start-up packages with access to state-of-the-art Genomics, Bioinformatics, and Flow Cytometry core facilities as well as BSL3/ABSL3 containment facilities within the institute. Interested candidates should submit curriculum vitae, a three-page description of their research program and the names of three references to:

Rafick Sekaly. Ph.D.
C/o Anne Gambardella
Scientific Director, Co-Director
Vaccine & Gene Therapy Institute Florida
11350 SW Village Pkwy 3rd Floor
Port St. Lucie, FL 34987

Review of applications will begin November 15, 2010, but will continue until the positions are filled. *The VGTI is an Equal Opportunity Institution committed to recruiting, hiring, and promoting qualified minorities, women, individuals with disabilities, and veterans.*

PROFESSOR of PRACTICE in Geographic Information Systems, Tulane University

The Department of Earth and Environmental Sciences seeks to fill a non-tenure-track, Professor of Practice position to teach courses in Geographic Information Systems (GIS), and to supervise the GIS computer lab. To be considered, the applicant must have experience using geologic, topographic, and geophysical raster and point cloud data sets. Expertise with ESRI products is required, including customization of GIS applications. We seek an individual possessing an enthusiastic dedication to teaching who is willing to make a long-term commitment to the department and the University. A Ph.D. is required at the time of appointment. The initial appointment will be for three years with the possibility of renewal after a performance review at the end of the second year. The deadline for applications is October 30, 2010, but the position will remain open until filled. Applications should include curriculum vitae, a statement of teaching interests and goals, and the names and contact information of at least three references, and should be sent to: **Dr. Stephen Nelson, Department of Earth & Environmental Sciences, Tulane University, 6823 Street Charles Avenue, New Orleans, LA 70118-5698. E-mail: snelson@tulane.edu** preferred. Further information about the department and University can be obtained at **website: http://tulane.edu/sse/eens.** *Tulane University is an Affirmative Action/Equal Opportunity Employer. Women and minorities are encouraged to apply.*

Call for application to the International PhD program of the Berlin-Brandenburg School for Regenerative Therapies (BSRT)

Berlin-Brandenburg School for Regenerative Therapies

Contact

Dr. Sabine Bartosch
Administrative Coordinator

Berlin-Brandenburg School
for Regenerative Therapies
Charité • Campus Virchow-Klinikum
Augustenburger Platz 1
13353 Berlin

Phone: +49 30 450 539418
Fax: +49 30 450 539918
E-Mail: info@bsrt.de
Web: www.bsrt.de

The international PhD program of the Berlin-Brandenburg School for Regenerative Therapies (BSRT) offers outstanding interdisciplinary training and research opportunities for students of natural, material and engineering sciences who want to pursue a career in the field of Regenerative Medicine and aim at translating their scientific discoveries into clinical applications.

The BSRT is a collaboration between the Charité - Universitätsmedizin Berlin, the Humboldt-Universität zu Berlin, the Freie Universität Berlin, the Technische Universität Berlin, the Universität Potsdam, Max Planck and Helmholtz Institutes and other high-ranking research institutions in Berlin and Brandenburg. This collaboration offers top class scientific training leading to the award of a Dr. rer. nat. or Dr.-Ing. degree for students with a Master's or equivalent degree in biology, biochemistry, bioengineering, veterinary medicine, chemistry, engineering or physics. It is not necessary to hold the Master's degree at the time of application but it must have been awarded prior to enrolment into the program. Students admitted to the PhD program will be supported by stipends.

The international PhD program of the BSRT comprises a series of courses, seminars and research projects designed to equip students with the essentials to become effective scientists in Regenerative Medicine. Interaction between biological, engineering and clinical research will be fostered through mutual supervision of each PhD project by top class researchers from different disciplines from academia and industry.

The next call for application will start on **1 November 2010**. Please use only the online application form provided on our website at www.bsrt.de where you can also find further information about the BSRT and the application procedure.

The BSRT is an Equal Opportunities Graduate school. We particularly encourage applications from women. We also welcome applications from disabled persons and the BSRT operates a policy whereby preference is given to disabled applicants who have qualifications equivalent to those of able bodied persons applying for the same position.

www.bsrt.de

Federal Ministry of Education and Research

bccn

"Bernstein Award" 2011

Young Scientists Research Award in Computational Neuroscience

The German Federal Ministry of Education and Research (BMBF) has established the "National Network for Computational Neuroscience" with six high-performing "Bernstein Centers for Computational Neuroscience" as the major structural elements.

The "Bernstein Award" is equipped with up to 1.25 Mio Euros in the form of a grant over a period of five years. It will be awarded to a highly qualified young researcher, considering the candidates' verifiable research profile in the field of Computational Neuroscience and the scientific concept for a future young research group. Young researchers can apply for their own position and group. The group funded by the "Bernstein Award" will become an integral part of the National Network for Computational Neuroscience. Future announcements of the "Bernstein-Award" are in the scope of the Ministry's planning.

The grant is provided for a scientific project of a young research group headed by a postdoc regardless of nationality. The project will be conducted at a German university or research institution – within or outside the Bernstein Centers. It is a prerequisite for funding that the university or research institution concerned employs the young researcher during the funding period and supports him/her with the basic equipment in terms of laboratory space and other infrastructure. A statement made to that effect by the receiving institution must be included with the project outline to be submitted.

Deadline for applications is May 20th, 2011.

For more detailed information about the "Bernstein-Award" including application conditions please visit

http://www.nncn.de
or
http://www.gesundheitsforschung-bmbf.de/en/2476.php

Focus on Germany

With the German Government now five years into a major program for investment in scientific research and innovation, we take a look at the new career opportunities that have opened up.

By Julie Clayton





"Politically, increasing diversity has become a goal. Our university has really tried, and has increased the number of foreign faculty significantly in the last couple of years."

—Jun Okuda

Growing up as a Japanese immigrant in Germany, **Jun Okuda** saw a very different world in the 1970s and '80s to the one he sees today. "The German system, both East and West, was very closed and they just would not accept foreign people in much."

Although Okuda had a happy childhood and teenage years, with no experience of discrimination, when it came to developing his scientific career his Ph.D. supervisor told him "you're Japanese, you may have no chance in the German academic system—it's too closed. There are very few foreign academic researchers."

Feeling impatient, Okuda resolved to "forget about Germany" and went to the United States. But by the '90s, it was clear that Germany was changing dramatically and was actively encouraging an influx of foreign researchers. Okuda was in New York when he was offered positions in Marburg and Mainz, and in 2003 he became chair of Organometallic Chemistry at the RWTH Aachen University.

"Politically, increasing diversity has become a goal. Our university has really tried, and has increased the number of foreign faculty significantly in the last couple of years," says Okuda.

This shift in attitude toward foreign scientists reflects not only the emergence of a more outward-looking German society since the reunification in 1990, but also Germany's desire to raise its scientific profile and modernize the science system. Formalized by the Federal Government in August 2006, the wide-reaching "High-Tech Strategy" forms part of a push to invest three percent of German gross domestic product in research and development by 2010, as per the EU's Lisbon Agenda. In November 2006, the Federal Ministry of Education and Research launched an initiative to "Promote Innovation and Research in Germany", including a commitment to invest approximately €15 ($19) billion. But to achieve this would require more recruitment and retention of both women and foreign workers if Germany were to stave off a predicted shortfall in skilled workers over the next couple of decades.

Another pillar of the new research and development policy is the Excellence Inititiative, a major funding package of €1.9 ($2.4) billion over five years. This was intended to shake up the university system and counteract the consistent failure for German universities to rank with the top 50 universities worldwide, despite Germany having Europe's largest national budget for R&D. Germany had no equivalent of Harvard or Oxford when it came to citations and other scores (see "A Strategy for the Future," *Science,* 2007; DOI: 10.1126/science.opms.r0700039).

The DFG and the German Council for Science and Humanities selected a total of 85 institutions to receive Excellence Initiative funds during 2006 and 2007. In total, 39 graduate schools (some newly created) received an average of €1 ($1.27) million per year over five years. They promised to improve the education and training of Ph.D. students and move away from the old system of poorly supervised research that could often take six or seven years to complete. Thirty-seven so-called 'Clusters of Excellence' received an average of €6.5 ($8.24) million per year for establishing new local research collaborations focused around specific topics and goals. And nine 'elite' universities were awarded up to €3.5 ($4.46) million per year for their new 'Institutional Strategies'. These included a range of improvements in teaching, research output, and competitiveness, as well as recruitment procedures for women and foreign researchers. The winners were the universities of RWTH Aachen, Free University of Berlin, Göttingen, Heidelberg, Karlsruhe, Freiburg, Konstanz, Munich LMU, and the Technical University of Berlin.

With just one year to go to the end of this first five-year phase, its impact is already apparent in many areas, with **continued »**

UPCOMING FEATURES

Diversity 2 (online only)—October 1

Top Employers Survey—October 8

Career Development Plan (online only)—December 3

CREDIT: (TOP) PHOTO ILLUSTRATION: *SCIENCE*; IMAGE: © ISTOCKPHOTO.COM/MAGUI80

abundant new research collaborations and networks. And according to the DFG, the Excellence Initiative has led to the creation of over 4,000 additional scientific jobs.

BREAKING FREE OF HIERARCHY

RWTH Aachen, for example, hosts three Clusters of Excellence, a graduate school, and has a range of programs around gender, diversity, and interdisciplinary research. Okuda is part of a Cluster of Excellence for the development of new "Tailor-Made Fuels from Biomass." It means that university researchers can break free of traditional vertical hierarchies to work together with others from different disciplines—engineering, aerodynamics, chemistry, and biotechnology—on a specific topic that is internationally competitive. Other Cluster members are the Fraunhofer Institute for Molecular Biology and Applied Ecology and the Max Planck Institute of Coal Research, Mülheim. "It has been difficult but interesting to work for one project. It has been an experience that was not possible before," says Okuda.

"The Excellence Initiative as a whole, but especially these Clusters have really made a difference in Germany because people had to think about what they could achieve together," agrees **Katja Sträßer,** an independent group leader at the Gene Center of the University of Munich. She participates in the Cluster known as the Center for Integrated Protein Science Munich (CIPSM), which investigates proteins at the level of biological systems, such as folding, transport, and interactions with nucleic acids. Other collaborators are groups from the Technical University of Munich, two Max Planck Institutes (Biochemistry and Neurobiology), and the Helmholtz Association's German Research Center for Environmental Health.

The Cluster has enabled strategic appointments to be made that boost expertise in particular areas. A major coup was the appointment of German scientist **Ulrike Gaul,** who, like Okuda, had spent several years in the United States before returning to Germany. An added bonus is the creation of new microscopy and high throughput sequencing facilities for the cluster.

Another outcome of the Excellence funding is a significant increase in the number of junior professorships on offer, permitting early independence and the availability of tenure-track positions. These are "new to the German system," says Sträßer. But the tendency for young Germans scientists to seek postdocs abroad, particularly in the United States and the United Kingdom, means that it can be difficult to persuade the best local candidates to stay. This raises the prospects for foreign researchers wishing to work in Germany (and returning Germans), although Sträßer emphasizes that nationality is not the issue: "It's a question of quality of incoming researchers, not their location."

TAKING FAMILIES INTO ACCOUNT

Sträßer also sees women scientists being taken more into consideration for top positions following concerns about women dropping out of science typically after their postdoc, which coincides with the time most people start a family. Appointment committees at LMU, for example, now **continued on p. 1682 »**

FEATURED PARTICIPANTS



CENTER FOR INTEGRATED PROTEIN SCIENCE MUNICH
www.cipsm.de/en/index.html

CHARITÉ MEDICAL SCHOOL IN BERLIN
www.charite.de/en/charite

DFG
www.dfg.de/en/index.jsp

FEDERAL MINISTRY OF EDUCATION AND RESEARCH
www.bmbf.de/en/14575.php

FRAUNHOFER INSTITUTE FOR MOLECULAR BIOLOGY AND APPLIED ECOLOGY
www.ime.fraunhofer.de/EN

FREE UNIVERSITY OF BERLIN
www.fu-berlin.de/en

GERMAN CANCER RESEARCH CENTRE
www.dkfz.de/index.html

GERMAN COUNCIL FOR SCIENCE AND HUMANITIES
www.wissenschaftsrat.de/1/home

HELMHOLTZ ASSOCIATION OF GERMAN RESEARCH CENTRES
www.helmholtz.de/en

THE LEIBNIZ ASSOCIATON
www.leibniz-association.eu

MAX PLANCK INSTITUTE
www.mpg.de/english/portal/index.html

MUNICH LMU
www.en.uni-muenchen.de/index.html

RWTH AACHEN UNIVERSITY
www.rwth-aachen.de/go/id/bdz

TECHNICAL UNIVERSITY OF BERLIN
www.tu-berlin.de

TECHNICAL UNIVERSITY OF MUNICH
portal.mytum.de/welcome

THE WELLCOME TRUST
www.wellcome.ac.uk

UNIVERSITY OF FREIBURG
www.uni-freiburg.de/start-en.html?set_language=en

UNIVERSITY OF GÖTTINGEN
www.uni-goettingen.de

UNIVERSITY OF HEIDELBERG
www.uni-heidelberg.de/index_e.html

UNIVERSITY OF KARLSRUHE
www.uni-karlsruhe.de/index_en.php

UNIVERSITY OF MANCHESTER
www.manchester.ac.uk

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Alexander von Humboldt
Stiftung/Foundation



Shine your light in Germany

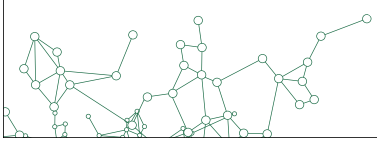
Profit from excellent conditions for research

The Alexander von Humboldt Professorships are Germany's way of creating a beacon effect and energising its research landscape. Every year, the Alexander von Humboldt Foundation is offering ten of the world's leading researchers up to five million EUR each to create new or consolidate existing internationally visible research focus areas at German universities.

Academics of all disciplines are eligible for an Alexander von Humboldt Professorship, provided that they are established abroad and recognised internationally as top-class researchers. They will be nominated by German universities – where appropriate in co-operation with non-university research institutions. Each Alexander von Humboldt Professorship will be sponsored for a period of five years on the premise that the university presents a convincing strategy to sustain the position once the funding period has come to an end. Accordingly, universities are asked to submit an implementation plan as part of the funding application. This will allow new, long-term research groups to be established, conducting cutting-edge (international) research. The German Ministry of Education and Research is supporting this programme.

Visit www.humboldt-foundation.de/ahp for more information. **Next closing dates for applications: 12 November 2010 and 13 May 2011**

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be part of a worldwide network.



Alexander von Humboldt Foundation
Jean-Paul-Str. 12
53173 Bonn
Germany
E-Mail: info@avh.de

www.humboldt-foundation.de



The change in attitude towards women scientists has been "really dramatic."
—Frauke Melchior

take family commitments into account when they are considering a candidate's list of publications. Rather than criticizing a woman scientist for too few papers in top journals, committees are now more likely to say "Yes, but she has also had two kids during that time so we should invite her and see what her plan is for the future." Sträßer herself has three young children.

"Women scientists are scrutinized and the more of us there are, the more it becomes normal rather than special—things are moving in the right direction," notes **Blanche Schwappach,** a Wellcome Trust Senior Research Fellow at the University of Manchester who is due to take up a full professorship and to become head of the department of Biochemistry I, Medical Faculty, at the University of Göttingen.

The change in attitude towards women scientists has been "really dramatic", agrees **Frauke Melchior,** full professor and member of the DKFZ-ZMBH Alliance (a collaboration between the German Cancer Research Center and the Center for Molecular Biology at the University of Heidelberg). She is also involved in Heidelberg's Cluster of Excellence on Cellular Networks. "I studied chemistry for my diploma and was constantly being told that I wouldn't get a position as a woman…a woman wouldn't have leadership capabilities because she wouldn't be respected." But with a working mother as a role model, Melchior had the confidence not to give up.

In her experience, women scientists are more likely to face "dual career" challenges, with partners who are also seeking high-level appointments. "The men still very frequently have a partner who has a job that is much more transferable. But if a university tries to recruit a successful female all of a sudden there is this problem that the spouse wants to have at least as good a job."

To address this issue, the University of Heidelberg and others have set up dual career services with Excellence Initiative funding. These provide advice and information about employment opportunities to encourage the partners of new professors, male or female, and are becoming increasingly popular. "We provide intensive counseling on the individual situation of the partners. We don't offer positions but we support them in finding a position, or refer them to a network of partners where they can get help," says **Christiane Stümke** project manager for Heidelberg's Dual Career Program, which has helped more than fifty couples in the past two years, around ten of whom have come from abroad.

PHASE TWO

Not everyone is happy with the results of the first round. Along with all the excitement, the Excellence Initiative has also attracted criticism for its emphasis on research. "People are worried that this might create a split so that low-profile universities do all the teaching and the high-profile ones are research universities," says Sträßer.

Looking ahead, universities and other institutions in Germany are now gearing up for a second phase of Excellence Initiative funding, which begins in November 2012. This will increase the Excellence Initiative Funding to €2.7 billion ($3.4 USD) over five years for new projects and renewal of existing projects for graduate schools, Clusters of Excellence, and Institutional Strategies. More broadly, the challenge for the universities will be to sustain the new initiatives supported by the first five year phase. "We have to see how it consolidates," says Schwappach. "The first round has unleashed a lot of potential but it's not clear how many initiatives will stay." Schwappach also points out that certain subject areas, including taxonomy or certain medical specialties such as radiology for children, are at risk of being "swept away" as universities play on their strengths in other areas.

WIDER OPPORTUNITIES

In addition to the Excellence Initiative, the German government provides a range of other funding streams including up to twelve years of support for Collaborative Research Centers (CRCs). Each CRC is hosted by a university, but involves collaboration with other institutions around specific research topics and can include institutions in neighboring countries.

One such CRC focuses on Biomechanics and Biology of Musculoskeletal Regeneration and is coordinated by the Charité Medical School in Berlin, and includes the **continued on p. 1684 »**

● USEFUL RESOURCES ●



RESEARCH IN GERMANY
www.research-in-germany.de

ALEXANDER VON HUMBOLDT FOUNDATION
www.humboldt-foundation.de

GERMANY RESEARCH FOUNDATION
www.dfg.de

DFG EXCELLENCE INITIATIVE
http://www.dfg.de/en/magazine/excellence_initiative/index.html

HELMHOLTZ ASSOCIATION OF GERMAN RESEARCH CENTERS
www.helmholtz.de/en/

FRAUNHOFER SOCIETY
www.fraunhofer.de/en/

MAX PLANCK SOCIETY
www.mpg.de/english/portal/index.html

LEIBNIZ ASSOCIATION
http://www.leibniz-association.eu/

GERMANY ACADEMIC EXCHANGE SERVICE – DAAD
www.daad.de

EURAXESS: RESEARCHERS IN MOTION
www.euraxess.de

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Julius-Maximilians-
UNIVERSITÄT WÜRZBURG

Universitätsklinikum Würzburg

Comprehensive Heart Failure Centre (CHFC) "Prevention of Heart Failure and its Complications"

The Comprehensive Heart Failure Centre (CHFC) is a central facility of both the University of Würzburg and the University Hospital commissioned to provide structures and expertise for multidisciplinary research, prevention and care of patients with heart failure and its complications. The Federal Ministry of Education and Research (BMBF) has announced funding from 11/2010. Research of the CHFC is organized in Project Areas centered around the following research themes:

- Project Area A: Advancement of diagnosis and management
- Project Area B: Healing, remodeling, protection
- Project Area C: Rare heart diseases and genetic principles
- Project Area D: Endocrine system and metabolism
- Project Area E: Cardio-renal cross talk
- Project Area F: Heart failure: emotion, cognition, cerebral dysfunction
- Project Area G: Advanced/end stage heart failure, tissue engineering and regenerative medicine
- Project Area H: Cardiovascular Imaging

From the time of funding the CHFC plans to establish
1. Clinical research positions
2. Junior Research Groups

SENIOR CLINICIAN SCIENTIST

A Clinical Research Centre comprising the CHFC Clinical Trial Unit, the CHFC Outpatient Department and the CHFC Centre for Biometry, Statistics and Study Coordination (CBSS) will be implemented to enhance top level translational, clinical and health care research. From the time of funding the CHFC plans to establish the position of a Senior Clinician Scientist.

Your Profile

- Accredited internist, comprehensive training in cardiology
- Scientific interest in the field of heart failure
- Adequate publication record and acquisition of 3r d party funding
- Profound experience in the design, conduct and analysis of patient-oriented research projects
- Mentoring ability and experience
- Ability and pleasure to work in a multidisciplinary team
- Identification with the goals of the CHFC

Your Task Area

- Coordination of the Clinical Trial Unit and CHFC Outpatient Department
- Supervision & quality control in the CHFC non-invasive diagnostic unit
- Close cooperation with the CBSS
- Design and implementation of new patient-oriented studies including RCTs according to GCP in collaboration with the CBSS
- Supervision of the clinical study team
- Resource allocation within the Clinical Trial Unit

Your Opportunities

- Multifaceted and responsible activity
- Function of a senior physician ('Funktionsoberarzt')
- Protected research time and development of own clinical projects
- Participation in the structured CHFC career development program (including State doctorate, if applicable)

CLINICAL TRAINING AND RESEARCH POSITIONS

In conjunction with the University Hospital the CHFC offers clinical resident and fellow positions for physicians. Positions may be shared by two applicants allowing for 50% protected research time and 50% clinical work to simultaneously enhance the development of both research and clinical skills. Physicians interested in both basic and clinical science may apply. The curriculum comprises excellent structured clinical training in cardiology and internal medicine covering all subspecialties; interdisciplinary clinical rotations are routinely performed to facilitate board exams in the expected time frame. It belongs to the goals of the CHFC to provide attractive career development and tenure track positions for excellent junior physician scientists. Salary will be according to TV-Ä.

JUNIOR RESEARCH GROUPS

Applications of young enthusiastic scientists of outstanding qualification in the field of cardiovascular research are invited. Support is granted to conduct independent research for a period of 5 years and includes:

- A position for the principal investigator
- Positions for one postdoctoral scientist, one PhD student and one technician
- Consumables and Equipment

Adequate space/ laboratory facilities will be available. The research focus of the junior research groups is not restricted but should complement the general objective of the CHFC and its Project Areas as described above. The CHFC career development plan offers tenure track positions for successful junior research group leaders. Selection for this position will be based on scientific qualification and publication records.

It belongs to the goals of the CHFC to increase the proportion of female scientists. Therefore, their applications are especially encouraged. Applications from severely handicapped persons with basically similar qualification will be given prioritiy.

For more information please contact

- C. Angermann (clinical research, angermann_c@medizin.uni-wuerzburg.de),
- S. Frantz (basic and translational research; frantz_s@medizin.uni-wuerzburg.de)
- S. Störk (clinical epidemiology; stoerk_s@medizin.uni-wuerzburg.de).

Applicants should send via e-mail a comprehensive CV, a summary of scientific achievements, activities and interests, and mentoring and teaching experience, a list of publications and the usual credentials including the names and addresses of 2 academic referees. Applicants for a Junior Research Group should add a short outline (12 pages) of their planned research activities.

Please send all documents embedded into one pdf-file or zip-file to the following address by October 15th, 2010:

Prof. Dr. G. Ertl • CHFC • Oberduerrbacher Str. 6 • D-97080 Würzburg • Germany
(Fax: ++49(0)931/201-639001 • e-mail: ertl_g@klinik.uni-wuerzburg.de).

FOCUS ON GERMANY
Science Careers online @sciencecareers.org



CRCs also enable universities to "hire more professors around a specific research topic in a more sustained way."
—Peter Fratzl

Julius Wolff Institute and the Department of Biomaterials at the Max Planck Institute of Colloids and Interfaces in nearby Potsdam. "The right people happened to be in the Berlin area," says **Peter Fratzl,** director-professor at the Institute.

The project, which includes funding for 12 research groups, pits together the skills and expertise of scientists across a range of disciplines: including biomechanics, surgery, genetics, and molecular biology.

In addition to emphasizing interdisciplinary research, CRCs also enable universities to "hire more professors around a specific research topic in a more sustained way," according to Fratzl. The result, he says, is "increased competence in that area."

Fratzl, who is Austrian, is aiming to recruit more foreign scientists with the offer of competitive research funding and a better working environment. He has already hired an Australian junior group leader in biomechanics, **John Dunlop,** for his department. After a Ph.D. in Grenoble, Dunlop was attracted to the MPI four years ago by the interdisciplinary work environment, as well as Berlin's culture and affordable living. "For me this is incredible," he says.

Matthew Harrington, a postdoc originally from the University of California at Santa Barbara, agrees, "The Berlin-Potsdam area is a very easy place to work in." He is a junior group leader (the equivalent of an assistant professor in the United States), and has not experienced any language barrier: "You have to force people to talk with you in German because in the lab everyone speaks English."

The German Federal Government and state governments (or Länder) also fund a Joint Initiative for Research and Innovation for the publicly funded non-university institutions, such as the Helmholtz Association, the Fraunhofer Society, the Max-Planck Society, and the Leibniz Association. They are now promised an increase of three to five percent to annual budgets every year from 2011 to 2015, plus additional funds for new programs that increase networking, international cooperation and recruitment, and partnerships between science and industry.

The Fraunhofer Society has already launched one new initiative, "Fraunhofer Attract", which has brought in 24 new young group leaders to build and run a group over five years, with a focus towards industrial product development. Of these, twelve came from abroad and five were women, which is an above-average recruitment of female scientists according to program director Raoul Klingler.

Similarly, the Helmholtz Association, which employs around 30,000 scientists in sixteen centers across Germany, has appointed 116 new "Young Investigators" (since 2006) to set up research groups, spending a total of €68 ($86.2) million. Approximately half of the group leaders have come from abroad and around forty percent were women (from an average of twenty to thirty percent of female applicants), according to press officer **Antonia Rötger.** The Helmholtz centers are geared towards strategic goals such as renewable energy, and are now increasing their networking with universities. The Association also has a new mentoring program for women researchers and management skills training that is open to researchers from other organizations.

TRAINING BEYOND BORDERS

In 2008 came Germany's new "Strategy for Internationalization of Science, Research, and Development" which aimed to promote cooperation beyond Germany's borders. One component is the DFG's International Research Training Programs, which is enabling Okuda at RWTH Aachen to bring young Japanese researchers, from the University of Osaka, to conduct research and participate in conferences and workshops. "We educate young researchers toward being more global scientists and managers. I think such experience in a completely different cultural setting is quite important. They have quite a culture shock, but they're young and adaptive, and learn very quickly how European society works," notes Okuda. In return, German researchers spend up to six months in Japan.

Meanwhile, the German Academic Exchange Service, DAAD, has €15 ($19) million to spend between 2010 and 2013 on the "International Doctoral Studies in Germany" program. This aims to make German universities more attractive to foreign students and to encourage German students to study abroad. Firstly, it funds the creation of structured Ph.D. programs more akin to U.S. graduate schools—but with less course work. Graduate students receive language classes and mentoring, and can complete their Ph.D.s within three years. Secondly, it funds binational cooperation between departments of universities in different countries. By the closing date, there were 86 applications for the two programs, with around thirty-five of these likely to be awarded, according to **Christian Schäfer**, the director. The initiative also serves as a boost to the universities that did not receive Excellence Initiative funding. "These incentives ensure that new developments cover the whole of Germany, so that these are not limited to the very big and rich institutions," he says.

With all the push towards internationalization, Okuda is convinced that prospects in Germany are "getting much better now" for foreign scientists wishing to take up senior positions. Nonetheless, the future is hard to predict. "Germany is in real turbulent times. I wouldn't dare to predict what it's going to be like in five years time."

Julie Clayton, a freelance science writer and journalist, works out of Bristol, UK.

DOI: 10.1126/science.opms.r1000095

Note: A response to the Focus on Spain feature, published 11 June, 2010, has been posted online. View it at dx.doi.org/10.1126/science.opms.r1000090.

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LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN
LMU ACADEMIC CAREER PROGRAM

LMU excellent

Ludwig-Maximilians-Universität (LMU) München is one of the leading research universities in Europe, with a more than 500-year-long tradition. It places particular emphasis on promoting young academics. As part of the "LMU Academic Career Program", LMU Munich will therefore award

10 Research Fellowships.





■ Applications are welcome from all candidates who have completed their doctoral studies within the last three years with outstanding results. Applicants must present an independent research project as part of their application. The project must be supported by a professor of LMU Munich. The fellows will become members of the Young Center of the Center for Advanced Studies and be able to make use of its services.







Endowment

Research fellows will receive an attractive salary according to the German "Tarifvertrag der Länder (TV-L)" (typically TV-L grade E 14). Applicants may apply for an additional start-up funding up to the amount of € 25,000 as well as for material and travel expenses of up to € 10,000 per year. In the first two years after the completion of their research fellowship, the fellows may be granted up to € 5,000 for continuing cooperation with LMU Munich. The fellowships are initially tenable for two years. They may be extended for a further two years on the basis of a positive academic evaluation.

Ludwig-Maximilians-Universität München is an equal opportunity employer committed to excellence through diversity and therefore explicitly encourages women to apply.

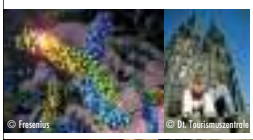
■ Closing date for applications is 15 November 2010.

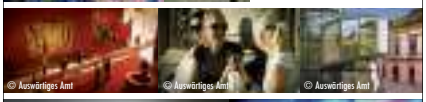
The prospective starting date of the fellowships is 1 June 2011.

For all information regarding your application please consult:
www.lmu.de/excellent/research-fellowships

www.research-in-germany.de

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Max Planck Institute for Biology of Ageing

MAX-PLANCK-GESELLSCHAFT

The Max Planck Institute for Biology of Ageing invites applications for a

Junior Group Leader in Biology of Ageing (W2)

We are seeking a highly talented, dynamic, independent, and motivated young biologist, who wishes to establish an independent Max Planck Research Group at our Max Planck Institute in Cologne. Successful candidates are free to develop their own, entirely independent, research programme using their own budget, which is of an amount that will fund research positions, consumables, equipment and travel grants. The position is open for five years with an option to extend it, to a maximum of 9 years. Further details can be found under http://www.mpg.de

The successful candidate will have an excellent scientific track record in a relevant research area, with clear evidence of independent research leadership, and a research interest in some aspect of the biology of ageing. The environment of the institute is international and the working language is English, so high proficiency in spoken and written English is essential.

The position is available immediately but the starting date is negotiable.

The new Max Planck Institute for Biology of Ageing was founded in 2008. It is part of a wider, and unique, network of several research institutions in the Cologne-Bonn area focused on research on ageing and ageing-related disease, constituting an expert and collaborative environment for research. Well equipped with state-of-the-art technology and excellent core facilities, the institute provides outstanding research opportunities for promising young scientists. Currently hosted in a new research institute building of the University of Cologne, the MPI will move into its own new, purpose-designed building in 2012.

The world feels at home in Cologne, Germany's oldest city, with more than 2000 years of history. The city owns innumerable cultural and historical treasures, world-famous museums and an active art scene. Life in Cologne is uncomplicated and vivacious. Due to Cologne's proximity to the German/Belgian/Dutch border, Paris, Amsterdam and Brussels a couple of hours away.

To apply for this position, please send a single pdf file including: (1) a cover letter explaining why you would like to join our Institute, (2) your Curriculum Vitae including your publication list, (3) a statement of scientific achievements, (4) a two page summary of your future research plans and (5) contact details for 3 academic references who are willing to write a reference letter on your behalf, and (6) up to three of your most important papers to the following email address: personal@age.mpg.de

Informal inquiries are welcome and should be sent to the same e-mail address. For further information see (http://www.age.mpg.de).

Max-Planck-Institut für Biologie des Alterns
Kennziffer 12/2010
Gleueler Str. 50a, 50931 Köln

CHARITÉ

The Julius Wolff Institute and the Center for Musculoskeletal Surgery invites jointly with the Berlin-Brandenburg Center for Regenerative Therapies (BCRT) applications for a

Professorship "Bioactive Implants for Musculoskeletal Regeneration"
Salary Group S-W2 BBesG

We seek to fill this position with an internationally outstanding scientist with a focus on basic research in bone, or cartilage regeneration. The centre fosters transfer of basic cell and molecular biology research into clinical applications. Candidates are expected to have an excellent track record in research and proven success in obtaining third-party funding. Preferably, the focus will be placed on the combination of cell, matrix biology and biochemical approaches with current treatment methods towards clinical applications. It is expected that the candidate aims for an active role in the Collaborative Research Centre SFB 760 "Biomechanics and Biology of Musculoskeletal Regeneration" and the Graduate School GSC 203 "Berlin-Brandenburg School for Regenerative Therapies". The professorship is integral part of the Julius Wolff Institute. The initial appointment is for five years with the possibility of further extension following successful evaluation. Written applications according to the format specified on the Internet-link **http://www.charite.de/en/charite/organization/careers/employment_market/internship/** should be submitted by **31.10.2010** to:

Prof. Dr. Annette Grüters-Kieslich, Dean
Charité - Universitätsmedizin Berlin
10098 Berlin

UNIVERSITY OF KONSTANZ

The University of Konstanz is one of the nine Universities of Excellence in the Federal Republic of Germany.

An opening for a

Post-Doc Position in Molecular Evolution, Genomics and Bioinformatics

(Salary Scale 13 TV-L/100 %)

is **available immediately** in the GeCKo – the Genomics Center of the University of Konstanz – http://cms.uni-konstanz.de/en/genomics-center/ .

Candidates should have a strong background in bioinformatics and/or molecular evolutionary biology and should be experienced in molecular biology techniques and/or computational biology.

For further information please visit our homepage: **http://www.uni-konstanz.de/stellen** .

Applications to position 2010/146 should be send as *one pdf file* to **axel.meyer@uni-konstanz.de** and include a CV, statement of research interests and email addresses of two references. Review of applications will **commence on October 15th, 2010**. Starting date is flexible.

Graduate Program in Computational Engineering Science

The Aachen Institute for Advanced Study in Computational Engineering Science (AICES) is a graduate school established within the frame of the German Excellence Initiative. AICES focuses on computational engineering science including such diverse fields as modeling and simulation; optimization; inverse problems and high-performance computing.
There is a limited number of openings in the AICES graduate program for exceptionally qualified students who hold a bachelor´s or master´s degree in engineering, natural science, mathematics, or computer science.
AICES offers a five-year path to the doctorate for students with bachelor's degrees, or a three-year path with master's degree, due to a novel advising and training concept. Admitted candidates receive tax-free stipends of 2000 € per month for the doctoral phase and 500 € per month for the master´s phase.

Graduate School AICES
RWTH Aachen University
Germany
www.aices.rwth-aachen.de
www.aices.rwth-aachen.de/admission

RWTH AACHEN UNIVERSITY

AICES



Photo:
Peter Winandy

EMBL

International PhD Programme

The European Molecular Biology Laboratory will award predoctoral fellowships in 2011 to university graduates wishing to study for a PhD at the EMBL in Heidelberg (Germany), at the EMBL research units in Hinxton (near Cambridge, UK), Hamburg (Germany), Grenoble (France) and Monterotondo (near Rome, Italy). Areas of study include:

- **Cell Biology & Biophysics**
- **Developmental Biology**
- **Genome Biology**
- **Structural & Computational Biology**
- **Bioinformatics**
- **Mouse Biology**

PhD work at EMBL is challenging, intense and intellectually demanding. We welcome applications from highly qualified students of all nationalities in the biological sciences, physics, chemistry, medicine, informatics, mathematics or engineering. Applicants must hold, or anticipate receiving before enrolment, a university degree that would formally qualify them to enter a PhD or equivalent programme in the country where the degree was initially obtained. All applications are evaluated solely on the basis of qualification and scientific potential.

The deadline for completion of the online application is December 13, 2010; registration deadline is December 1, 2010. Successful candidates are awarded fellowships in early March 2011 to start work at EMBL any time after the interviews and by latest October 2011. The duration of a fellowship is 3 1/2 years. Following completion of their PhD thesis, students have the opportunity to obtain joint PhD degrees between EMBL and one of its partner universities in Europe. Alternatively, students can opt to defend their thesis at a recognised university of their choice.

A brochure is also available on request from the: **Dean of Graduate Studies, Dr. Helke Hillebrand, EMBL, Postfach 10 22 09, D-69012 Heidelberg, Germany (Tel: +49 6221 387 8612, Fax: +49 6221 387 8400, e-mail: predocs@embl.de)**.

All applications have to be made online at:
www.embl.org/phdprogramme

LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

FACULTY OF PHYSICS

The **Faculty of Physics** of the Ludwig-Maximilians-Universität München (LMU) invites applications for the position of a

Full Professorship (W 3) for Experimental Physics – Medical Physics.

The successful candidate will have to represent the entire field of Experimental Physics in research and teaching at the university.

He/she is expected to have demonstrated excellence in research and scholarship in one or more areas of medical physics, preferably in the physics and/or technology of either biomedical imaging or radiotherapy. The faculty is searching broadly, and outstanding candidates working in any branch of medical physics will be given serious consideration.

The professorship is embedded in the cluster of excellence *Munich Centre for Advanced Photonics* (MAP, www.munich-photonics.de) and the *Centre for Advanced Laser Applications* (CALA), which is under construction. MAP pursues a highly integrated research program by interlinking physics and life sciences to advance cutting-edge laser technologies and their medical applications. CALA will provide a world-leading infrastructure of laser-based brilliant X-ray sources for early detection of cancer and degenerative diseases and particle sources for cancer therapy. The opening for the new chair emerges in this environment from the vision of bridging physics and medical sciences in research and teaching.

The successful candidate is expected to develop an interdisciplinary research program connecting state-of-the-art physical technologies with medical applications in collaboration with groups active in MAP and CALA and to take an active role in establishing a graduate program in medical physics.

Prerequisites for this position are a university degree, a doctoral degree, teaching skills at university level and a post-doctoral degree (Habilitation) or equivalent qualification that may have been gained outside the university or may consist in a junior professorship.

Candidates for this position should be aged 52 or under at the time of appointment. Exceptions thereto may be considered in the case of outstanding candidates.

The LMU is an equal opportunity employer and aims to increase the number of female faculty members. Therefore, applications from female candidates are explicitly encouraged.

Disabled candidates with essentially equal qualifications will be given preference.

The LMU supports dual career couples.

Applications including a curriculum vitae, academic records, a list of publications and invited lectures, a summary of planned research activities should reach the **Dekanat der Fakultät für Physik der Ludwig-Maximilians-Universität München, Schellingstr. 4, 80799 München, Germany,** by **November 30, 2010**.

Julius-Maximilians-
UNIVERSITÄT
WÜRZBURG

W2 and W3 research professorships "Comprehensive Heart Failure Center Würzburg" - Deadline: 22 October 2010

The Comprehensive Heart Failure Center of the University Hospital Würzburg aims to install four research professorships for 5 years starting 1 November, 2010. The research professorships will be appointed in the context of creating the BMBF-supported Integrated Center for Research and Treatment (IFB) with its theme `Prevention of Heart Failure and its Complications´.

1.) W3 (Full) Professorship "Molecular and Cellular Imaging"

The applicant is expected to implement a chair for experimental cardiovascular imaging. The department should integrate imaging for experimental and clinical heart failure research with different methods (MRT, CT, PET/SPECT, microscopy). The applicant is supposed to be a doctor of medicine, chemistry, or physicist. The applicant should have an excellent publication track and have raised significant amounts of external funds in cardiovascular imaging. A research focus on heart failure in the IFB is mandatory.

2.) W2 Research Professorship "Translational Research"

Applicants are expected to represent heart failure in research, teaching and patient care. The applicant should be a clinician trained in cardiology with experience in basic and translational research with a focus on animal models of heart failure. The applicant should have a broad publication record in basic cardiovascular science of heart failure and should have raised significant amounts of external funds. The applicant should identify her-/himself with the principles of the IFB.

3.) W2 Research Professorship "Epidemiology of Heart Failure"

Applicants are expected to represent clinical epidemiology of heart failure in research and teaching. The applicant should be a clinician trained in cardiology and clinical epidemiology. Cooperation with the chair of epidemiology and biometry is expected as are teaching activities for the new MD/PhD tracks `Clinical Research´. The applicant should have extensive experience in the conduct of clinical studies in heart failure and should be familiar with modern biomaterial banking concepts. The applicant should have an excellent publication track and have raised significant amounts of external funds in clinical heart failure research.

4.) W2 Research Professorship "Cardiovascular Genetics"

Applicants are expected to set up cardiovascular genetics in the IFB. The applicant should have experience in complex and in mono-genetic diseases with its respective methodology (genome wide association studies, copy number variation, sequencing). It is expected that the applicant has extensive experience in cardiovascular genetics, a broad publication and substantial external funding record. A research focus on heart failure is mandatory, as is the implementation of the interdisciplinary Core Facility Genetics in accordance with participating partners (Institute of Clinical Biochemistry and Pathobiochemistry, Psychiatry, Human Genetics).

All applicants have to fully identify themselves with the underlying research theme `Prevention of Heart Failure and its Complications´. Requirements include: a university degree, a thesis ("Promotion"), "Habilitation" or an equivalent level of scientific qualification and academic teaching ability. Würzburg University is committed to increase the number of women employed in academic research. We therefore particularly encourage female candidates to apply. Applications from disabled people of equal aptitude will be favoured. Applications from exceptional scientists referring to the above mentioned positions including full CV, publication list and documentation of teaching experience should be sent no later than 22 October, 2010 to: the Dean of the Medical Faculty of the University of Würzburg, Professor Dr. M. Frosch, Josef-Schneider-Strasse 2, D-97080 Würzburg. The legally binding German version of this announcement is published in "Deutsches Ärzteblatt" on 1 October, 2010.

UNIVERSITÄTSmedizin.
MAINZ

University Medical Center
of the Johannes Gutenberg University Mainz
Institute of Physiological Chemistry

POSTDOCTORAL / GROUP LEADER Position in Electrophysiology

Using genetic models in mice, our group investigates mechanisms underlying various behaviours and the involvement of the endocannabinoid system. We seek a highly motivated and innovative researcher with the initiative and the ability to establish his/her own research project and secure external funding as well as develop a close collaboration within the institute and with other research groups on the university campus. We offer state-of-the-art laboratories, a stimulating environment, and a well-equipped animal facility in addition to financial support. The applicant is expected to contribute to the teaching of medical students in biochemistry and molecular biology, with the potential for habilitation. The knowledge of the German language is required. The position (TVL-13) is for three years with the option for extension.

For further information and to apply, please contact **Dr. Beat Lutz** (e-mail: blutz@uni-mainz.de), before **1 December 2010**.
For application, include CV, list of publication, summary of research achievements and future projects, and three letters of references.

Bioengineering Faculty

The George Mason University, Volgenau School of Engineering is seeking faculty members, especially at the Assistant and Associate Professor levels, to continue building a new program in bioengineering that now offers a B.S. degree. Each successful applicant will receive a tenure-track appointment in one of the Volgenau School departments (http://ite.gmu.edu/), and will be expected to shape bioengineering through both research and teaching. George Mason University is an innovative, entrepreneurial institution with four campuses in the greater Washington, D.C. area. Potential interactions with government agencies and medical institutions abound.

Candidates must have earned a doctorate in bioengineering, biomedical engineering, or a closely related field. Preference will be given to applicants with a strong potential for interaction with current faculty members, especially in integrating measurement and computational approaches. Neuroengineering, bioimaging, rehabilitation engineering, biosensors and computational bioengineering are of special interest.

Applicants should submit a cover letter with contact information for at least three references, a CV, and a statement of research and teaching interests for **position F9404z at http://jobs.gmu.edu/**. Review of applications will begin immediately, and continue until the positions are filled.

See http://bioengineering.gmu.edu for an overview of bioengineering at Mason. Questions should be directed to Prof. Peter Katona (pkatona@gmu.edu).

EOE

GEORGE MASON UNIVERSITY

BC Cancer Agency
CARE & RESEARCH

An agency of the Provincial Health Services Authority



The **BC Cancer Agency (BCCA)** is seeking a research leader of international profile with a record of scientific excellence to lead the world-class research activities of the BC Cancer Agency, as the Vice President Research. The BC Cancer Agency is affiliated with UBC and co-hosts the development and conduct of academic oncology in BC. The position entails responsibility for visionary, strategic leadership and overall direction, coordination and oversight of cancer research at the BC Cancer Agency. The BC Cancer Agency is a provincial organization with a provincial mandate for cancer control and undertakes world-class research in many disciplines, utilizing many technologies within laboratory-based and translational research, clinical trials and population oncology.

The appointee will also serve as Associate Dean, Research within the Faculty of Medicine at the University of British Columbia (UBC). In this endeavor, this individual will work with the Executive Associate Dean, Research, and Associate Deans, Research of the lower mainland Health Authority Research Institutes (Vancouver Coastal Health Research Institute, Child and Family Research Institute and Providence Health Care Research Institute) for both defining and facilitating the health research strategy for the Faculty of Medicine (FoM), province wide.

Vice President Research – BC Cancer Agency
Assistant Dean, Research – University of British Columbia

Regular Full-time *Vancouver, British Columbia*





The Vice President Research has dual responsibility. The incumbent will report jointly to the President BC Cancer Agency and, within the Faculty of Medicine, this individual will report to the Executive Associate Dean, Research and through this position, is accountable to the Dean of the Faculty of Medicine.

The ideal candidate will have education and experience equivalent to a PhD and/or MD in a relevant discipline as well as bring strong administrative and interpersonal skills to the position. Key attributes of the successful applicant will include scientific excellence, innovation, collaborative relationships with local, national and international partners, promotion of 'team research' and the ability to lead a strategically focused program of excellence in scientific discovery and translational research. The individual will have a track record of significant research achievement, demonstrated excellence in teaching, and be eligible to hold a clinical faculty appointment or a full-time appointment at the rank of Professor in the UBC Faculty of Medicine. An appropriate academic department in the Faculty of Medicine will be identified at the time a successful candidate is selected. Salary will be commensurate with experience and qualifications. The expected start date for the position is January 3, 2010 or upon a date to be mutually agreed. The term would be for 5 years initially.

Application letters, accompanied by a detailed curriculum vitae, a teaching dossier, and names of three references should be submitted to: **Carol Jeffs, Recruitment Advisor, #800 – 1441 Creekside Dr, Vancouver, BC V6G 4S7 cjeffs@phsa.ca • 1-604-875-7215 (P) • 1-604-875-7253 (F).** For more information, please contact **Dr. David Levy, President, BC Cancer Agency** at **604.675.8100** or **dlevy@bccancer.bc.ca**

Applications will be accepted until **October 12, 2010.**

For more information about the Agency that you are interested in, please visit the website at: **www.bccancer.bc.ca**

For more information on all that the PHSA has to offer, please visit: **http://careers.phsa.ca**

The BC Cancer Agency is committed to reducing the incidence of cancer, reducing the mortality from cancer, and improving the quality of life of those living with cancer. It provides a comprehensive cancer control program for the people of British Columbia by working with community partners to deliver a range of cancer services, including prevention, early detection, diagnosis and treatment, research, education, supportive care, rehabilitation and palliative care. It operates five regional cancer centres in Surrey, Kelowna, Vancouver, Vancouver Island, and Abbotsford, with a sixth centre scheduled to open in Prince George in 2012. The BC Cancer Agency's Research Centre conducts research into the causes and cures for cancer.

BCCA is an agency of the **Provincial Health Services Authority (PHSA)** which plans, manages and evaluates specialty and province-wide health care services across BC. ***PHSA embodies values that reflect a commitment to excellence. These include: Patients first • Best value • Results matter • Improvements through knowledge • Open to possibilities.***

UBC

Provincial Health Services Authority
Province-wide solutions. Better health.

BC Cancer Agency and UBC hire on the basis of merit and are committed to employment equity. All qualified persons are encouraged to apply. UBC is strongly committed to diversity within its community and especially welcomes applications from visible minority group members, Aboriginal persons, persons with disabilities, persons of any sexual orientation or gender identity, and others who may contribute to the further diversification of ideas. However, Canadian and permanent residents of Canada will be given priority.

www.phsa.ca **www.bccancer.bc.ca**

Science Careers online @sciencecareers.org

Burnett School of Biomedical Sciences College of Medicine

Faculty Positions in Four Areas: Cancer, Cardiovascular and Metabolic Diseases, Infectious Diseases and Neurodegenerative Diseases



University of Central Florida is expanding its Biomedical Research and Education Program into a new 198,000sq.ft. Burnett Biomedical Science building in the new medical campus. We seek outstanding scientists working on molecular, cellular, physiological, biochemical, or pharmacological approaches to study important problems with relevance to cancer, cardiovascular and metabolic diseases, infectious diseases and neurodegenerative diseases. Faculty at Assistant, Associate or Full Professor level will be considered. Successful applicants must hold an earned doctorate in a discipline appropriate to the school and will be expected to establish a well funded research program, contribute to teaching, and actively participate in MS and PhD programs.

Competitive salaries, startup funds, new laboratories, transgenic animal facilities and access to the new multimillion dollar shared core instrumentation facilities will be provided. Medical campus is part of multibillion dollar biomedical cluster that includes the Burnett School of Biomedical Sciences, the Medical School, Sanford-Burnham Medical Research Institute, Veterans Administration Hospital and Nemours Children's Hospital. Burnett School researchers will have access to the extensive core facilities in the adjacent Sanford-Burnham Medical Research Institute.

The University of Central Florida is the nation's third largest university and ranks third in innovation and patents. It is located in Orlando, a dynamic and progressive metropolitan region, a major player in high-tech industry, and adjacent to a top ranked Research park and a great place to live and work.

Review of candidates will begin on November 1, 2010. Please apply specifying your area of interest, a curriculum vitae, a two page summary of research plans and contact information for three or more references to **biomed@mail.ucf.edu** .

University of Central Florida

The University of Central Florida is an equal opportunity, equal access, and affirmative action employer. As a member of the Florida State University System, all application materials and selection procedures are available for public review.

FACULTY POSITION IN CHEMISTRY

Department of Chemistry Arts and Science

The Department of Chemistry at New York University (NYU), located in Greenwich Village in the heart of Manhattan, invites applications for a faculty position in chemical biology as part of a new Biomedical Chemistry initiative. All ranks will be considered, but applications from junior faculty candidates are preferred. The anticipated start date is September 1, 2011, pending budgetary and administrative approval. The Department of Chemistry at NYU is implementing a significant growth plan, including the Biomedical Chemistry initiative, creation of the Molecular Design Institute, and the recent hire of five senior and two junior faculty members.

Applicants should have an outstanding record of research and a commitment to teaching. Applications should include a curriculum vitae, a list of publications, statements of future research and teaching plans, and three reference letters. *Application review will begin* ***October 15, 2010****; completed applications received by that date will receive full consideration*. Applications should be sent through our web-based submission system, PeopleAdmin: **www.nyuopsearch.com/applicants/Central?quickFind=50607** If you have any questions about this, please send an email to **chemistry.search@nyu.edu**. NYU encourages applications from women and members of minority groups.

NEW YORK UNIVERSITY

NYU is an Equal Opportunity/Affirmative Action Employer.

Tenure Track Faculty Position in Proteomics Sloan-Kettering Institute

The Sloan-Kettering Institute, Memorial Sloan-Kettering Cancer Center seeks to fill a tenure-track faculty position in mass-spectrometry-based proteomics. The appointment can be at the Assistant, Associate, or Full Member level, depending upon the experience of the successful candidate. We are interested in individuals with outstanding research accomplishments in proteomics, who wish to address significant biological problems broadly relevant to cancer and/or develop new methodologies. Faculty will be eligible to hold graduate school appointments in the Gerstner Sloan-Kettering Graduate School of Biomedical Sciences, the Weill Graduate School of Medical Sciences of Cornell University, as well as the Tri-Institutional MD/PhD Training Program and the Tri-Institutional Training Programs in Chemical Biology and Computational Biology. Applicants should have a Ph.D. or MD degree and postdoctoral research experience.

The Sloan-Kettering Institute offers a unique and highly collaborative research environment with programs in Pharmacology, Immunology, Chemistry, Molecular Biology, Computational Biology, Cell Biology, Developmental Biology, Structural Biology, Cancer Biology and Genetics. The adjacency of world-renowned clinical programs in cancer research, treatment and prevention offers unique opportunities for translation of basic discoveries.

The deadline for applications is **November 15, 2010**. Interested candidates should visit **http://facultysearch.ski.edu** to access the on-line faculty application. Please visit the site as soon as possible, as it contains important information on the required application materials, including deadlines for submission of letters of reference. Inquiries may be sent to **Marie Aiello at aiellom@mskcc.org or to Dr. David Scheinberg, Chairman, Molecular Pharmacology and Chemistry Program at d-scheinberg@ski.mskcc.org**. MSKCC is an equal opportunity and affirmative action employer committed to diversity and inclusion in all aspects of recruiting and employment. All qualified individuals are encouraged to apply.

Memorial Sloan-Kettering Cancer Center

www.mskcc.org

THE UNIVERSITY OF ARIZONA

TRANSLATIONAL CARDIOVASCULAR BIOLOGY FACULTY POSITION

The University of Arizona, in Tucson, seeks to hire a tenure-eligible physician-scientist at the Assistant or Associate Professor level whose research focus lies in any area of Cardiovascular Biology. Candidates with research interests related to Sudden Cardiac Death may be eligible to hold a Sarver Heart Center Endowed Chair.

The position will reside in the new Clinical and Translational Sciences Institute (CaTS-1) under the umbrella of the BIO5 Institute. The primary College of Medicine Clinical Academic Department and a joint appointment in a Basic Science Department will be chosen to match with the expertise of the applicant.

The successful candidate will be expected to develop or to bring an independent research program that spans basic and clinical cardiovascular biology, thereby strengthening our translational efforts at the University. Research time (75%) will be protected by the BIO5 Institute **(http://bio5.org)** for the first five years of this appointment in order to facilitate development of the applicant's translational research program. Candidates with M.D./Ph.D. degrees are particularly encouraged to apply.

Requirements: If applying at the Associate Professor Level, the applicant is expected to have a solid record of Federal and/or other research funding, as well as significant publications.

To apply visit: **www.uacareertrack.com, Job Number 45254.**

For additional information, contact **Search Committee Chairs, Carol C. Gregorio, Ph.D. at gregorio@email.arizona.edu** or **Gordon A. Ewy, M.D. at GAEWY@aol.com.**

The University of Arizona is an AA/EOE – M/W/D/V Employer.

UNC CHARLOTTE

Chair, Department of Biology

The University of North Carolina at Charlotte, ranked by "US News & World Report" in the top ten "Up and Coming Schools" among National Universities, invites applications and nominations for the Chair of the Department of Biology (http://biology.uncc.edu). The chair is expected to play a significant role in facilitating the Department's contribution to the University goal of becoming a leading research university, while maintaining the primary commitment of providing educational opportunities for qualified students of diverse backgrounds through informed and effective teaching.

Qualifications: This tenured position is open to applicants with credentials and experience appropriate for a full professor. Candidates must possess: (1) a doctoral degree in the biological sciences; (2) excellent leadership and interpersonal skills; (3) demonstrated ability to work across disciplines and lead a rapidly growing, diverse department dedicated to teaching and research excellence; (4) a distinguished record of teaching, scholarly achievement, and externally funded research; and (5) the ability to foster the development and implementation of a comprehensive plan that articulates an exciting, dynamic, and strategic vision for the Department's future. Previous administrative experience is highly desirable.

Responsibilities: The selected candidate will have the ability to: (1) work with faculty and students from multiple disciplines; (2) attract and retain top-tier faculty and promote professional development; (3) guide existing degree programs at both the undergraduate and graduate levels; (4) advance the Department's and University's commitment to research in the biological sciences, teaching excellence, and community-based scholarship and outreach; and (5) support and promote diversity among students and colleagues. The preferred starting date is July 1, 2011.

The Department: The Department has 30 full-time and 4 research faculty members from a variety of disciplinary backgrounds, 7 staff members, and a student population of over 1000 undergraduates, over 30 Master's students, and approximately 30 doctoral students. Our mission is to create, extend and disseminate basic and applied knowledge of the biological sciences through scholarly research, teaching, and service. To this end, the Department has developed strong collaborative interactions with the Bioinformatics, Nanoscale Science, Infrastructure and Environmental Systems, and Engineering PhD programs within the University, as well as with the Carolinas Medical Center, the Duke University Comprehensive Cancer Center, and with several marine laboratories, including the UNC Wilmington Center for Marine Science Research, the NC State Center for Marine Sciences and Technologies, and the UNC Institute of Marine Sciences. The Department and College are strongly committed to interdisciplinary research and teaching, supporting a range of interdisciplinary programs from minors to doctoral programs.

The University: UNC Charlotte, the only doctoral research university in the Charlotte metropolitan area and the fourth largest of 17 institutions in the University of North Carolina system, is a dynamic research institution with approximately 900 full-time faculty and offers 18 doctoral programs, 62 master's degree programs and 90 programs leading to bachelor's degrees. Fall 2010 enrollment exceeds 25,000 students, including almost 5,200 graduate students, and is expected to grow to 35,000 by 2020.

Applications and Nominations

Candidates must apply online at **http://jobs.uncc.edu**, position number: **6139**. Please include a letter that clearly describes your leadership vision and includes philosophy statements for leadership, research, and teaching, a current and complete vita, contact information for three references; and three representative publications. Screening of applications will begin October 4, 2010, and continue until the position is filled.

For more information, confidential inquiries, or nominations, please contact either **Search Chair Dr. Robert G. Wilhelm**, (**704-687-8428, rgwilhel@uncc.edu**) or **George Rent of Rent Consulting Group** (**704-366-2388**, **grent@rentconsultinggroup.com**), the search firm assisting with the search.

The University of North Carolina at Charlotte is an EOE/AA employer and an ADVANCE Institution that strives to create an academic climate in which the dignity of all individuals is respected and maintained. Therefore, we celebrate diversity that includes, but is not limited to ability/disability, age, culture, ethnicity, gender, language, race, religion, sexual orientation, and socio-economic status.

RENT CONSULTING GROUP, LLC

Science Careers online @sciencecareers.org

NEW YORK UNIVERSITY

ASSISTANT PROFESSOR OF MOLECULAR AND CELLULAR BIOLOGY

Department of Biology
Arts and Science

As part of a multi-year hiring plan, New York University's Department of Biology (**http://biology.as.nyu.edu/**) invites applications for a tenure-track Assistant Professor in the area of molecular and cellular biology to begin September 1, 2011, or as negotiated, pending budgetary and administrative approval. Candidates investigating molecular & cellular mechanisms underlying cell growth, differentiation and/or transformation are especially encouraged to apply. Strong interactions will exist with faculty in the Center for Genomics and Systems Biology and the Center for Developmental Genetics. These Centers within the Department of Biology offer an outstanding and collegial research environment.

Candidates will be expected to have or develop active, externally funded research programs and to participate in the Department's teaching activities at both the undergraduate and graduate levels. The candidate will specifically be expected to teach in the Department's core undergraduate course "Molecular and Cellular Biology".

Please apply online at the New York University Department of Biology website (**http://biology.as.nyu.edu**), via the "**Faculty Recruitment**" link. Be sure to include a cover letter, research statement, curriculum vitae, and three letters of reference by ***November 16, 2010***. Applications received before this date will be guaranteed full evaluation.

NYU is an Equal Opportunity/Affirmative Action Employer.

The University of Texas at Austin

Cancer Molecular Biology Position

The Institute for Cellular and Molecular Biology

The Institute for Cellular and Molecular Biology, Alan Lambowitz, Director, invites applications for a tenure-track/tenured position in cancer molecular biology. Academic appointments at the level of Assistant, Associate, or Full Professor will be in the section of Molecular Genetics and Microbiology in the College of Natural Sciences. Candidates should have an outstanding record of research productivity and a research plan that utilizes molecular and biochemical approaches to address important problems in cancer biology. Areas of particular interest include but are not limited to DNA damage responses, genome instability, post-translational regulatory mechanisms, chromatin, and small regulatory RNAs.

Building on a strong existing faculty, the Institute has recruited more than 50 new faculty members over the past ten years. In addition to its highly interactive and interdisciplinary research environment, the Institute provides administrative and financial support for the Graduate Programs in Cell and Molecular Biology, Microbiology, and Biochemistry, and state-of-the art core facilities including DNA sequencing, mass spectrometry, electron and confocal microscopy, DNA microarrays, robotics, mouse genetic engineering and Next-Gen sequencing. An MD-PhD program with the UT Medical Branch and the new Dell Pediatrics Research Institute enhance the environment for basic Biomedical Research.

Austin is located in the Texas hill country and is widely recognized as one of America's most beautiful and livable cities.

Please send a single PDF file containing your curriculum vitae, summary of research interests and names of three references before November 1, 2010 to: **mgm_search@biosci.utexas.edu.** References may also send their letters directly to the same email address.

Homepages • http://www.icmb.utexas.edu • http://www.biosci.utexas.edu/MGM •

The University of Texas at Austin is an Equal Opportunity Employer.
Qualified women and minorities are encouraged to apply; a background check will be conducted on applicant selected.

The University of Colorado Endowed Chair Professorship in Biochemistry

The University of Colorado Boulder is seeking applications and nominations for the Cech-Leinwand Endowed Chair in Biochemistry. The scientist will hold the rank of Professor in the Department of Chemistry and Biochemistry. It is expected that the successful candidate will be an internationally recognized, outstanding scientist with a track record of well-funded research, broadly cited publications and will have demonstrated success in teaching, graduate training, and service to the academic community.

The successful candidate will be housed in a new building designed specifically to foster collaborative research in the field of biotechnology and provide an interdisciplinary environment for faculty and students. The research space will be shared with world-class scientists conducting multidisciplinary research in systems biology, biochemistry, bioorganic chemistry, computational biology, and biomedical engineering. It is expected that the successful candidate will conduct collaborative research with colleagues in order to solve complex biomedical problems, requiring the expertise from several disciplines. Applications and nominations are accepted via e-mail to **diane.hager@colorado.edu**. Applications must include a cover letter, a statement of current and future research interests, a curriculum vitae including previous and current funding, and each candidate should ask at least three qualified scientists to send letters of recommendation supporting the application. Review of applications will begin on **November 1, 2010**. Applications will be accepted until the position is filled.

The University of Colorado does not discriminate in any condition of employment on the basis of race, color, national origin, sex, age, disability, creed, religion, sexual orientation, or veteran status. Equal Employment Opportunity (EEO) shall apply to all terms, conditions, and privileges of employment, including hiring, probation, testing, training and development, promotion, transfer, compensation, benefits, educational assistance, discipline, termination, layoffs, social, cultural and recreational programs, and retirement. CU-Boulder is committed to making employment decisions in all job titles based on valid job requirements, without regard to race, color, national origin, sex, age, disability, creed, religion, sexual orientation, or veteran status.

The University of Colorado at Boulder is committed to diversity and equality in education and employment. See **www.colorado.edu/ArtsSciences/Jobs/** for full job description.

STANFORD UNIVERSITY

STANFORD UNIVERSITY
DEPARTMENT OF CHEMICAL AND SYSTEMS BIOLOGY

The Department of Chemical and Systems Biology at Stanford University School of Medicine invites applications for two tenure-track positions at the ASSISTANT PROFESSOR level. We are particularly interested in candidates with a strong interdisciplinary record in the broad areas of chemical biology, systems biology, and/or cellular and molecular biology in normal and disease states. Stanford offers an outstanding environment for creative interdisciplinary biomedical research. The main criterion for appointment in the University Tenure Line is excellence in research and teaching.

Candidates should have a Ph.D. and/or M.D. degree and postdoctoral research experience. Candidates should send curriculum vitae, a description of future research plans and the names and addresses of three potential referees by **November 1, 2010** to:

James Ferrell, Professor and Chair
c/o Jean Kavanagh, FAA
Department of Chemical and Systems Biology
269 Campus Drive, CCSR Bldg Room 4145A
Stanford University School of Medicine
Stanford CA 94305-5174

Stanford University is an Equal Opportunity Employer and is committed to increasing the diversity of its faculty. It welcomes nominations of and applicants from women and minority groups, as well as others who would bring additional dimensions to the university's research, teaching, and clinical missions.

National Oceanography Centre
NATURAL ENVIRONMENT RESEARCH COUNCIL

School of Ocean and Earth Science

The School of Ocean and Earth Science forms the University component of activity at the National Oceanography Centre, Southampton. The Centre represents an unparalleled investment in marine and Earth science and technology in the UK. It opened in 1995 in a purpose-built waterfront campus in a collaboration between the University of Southampton and the Natural Environment Research Council (NERC). The Centre houses around 500 staff. The School of Ocean and Earth Science comprises about 120 staff, 500 undergraduate and 200 postgraduate students. The School forms part of the new Faculty of Natural and Environmental Sciences, along with the Schools of Chemistry and Biological Sciences.

We have a diverse range of excellent laboratory and seagoing facilities. The Centre provides a unique intellectual environment for conducting world-class research and for training the next generation of scientists for a wide range of careers. The School is in an exciting phase of recruitment in which we plan to make multiple academic appointments over the next few years. We are therefore interested to receive expressions of interest from high-quality individuals in all areas of Ocean and Earth science in addition to the areas specified below. Chair (full professor) candidates should be established international leaders. We will also consider appointments at Readership (associate professor) level. At Lecturer level (equivalent to tenure-track assistant professor), we are interested in recruiting early-career individuals with growing international reputations for research excellence and with the ambition to become international leaders in their fields.

Chair in Organic Geochemistry

From £58,092 pa | Ref: 4816-10-F

We seek an international leader in the field of organic geochemistry to complement our strong existing research groups in palaeoceanography, geochemistry and ocean biogeochemistry. Areas of interest include proxies for palaeoclimate reconstruction, sedimentary and water column organic biogeochemistry and/or the preservation and transformation of organic matter (including petroleum systems). Within the Centre, we have recently been awarded funding to renew and expand analytical facilities (including IT-LC-MS, GC-IR-MS, HR-ICP-MS, MC-ICP-MS, CHN and TOC-TDN instruments). Further facilities are available in the School of Chemistry. Excellent opportunities exist for collaboration with local NERC colleagues and with other Schools and Research Centres (such as Centre for Proteomic Research) in the University. You will be expected to contribute to research-led education in appropriate areas of earth and/or ocean science.

Chair in Marine Biology

From £58,092 pa | Ref: 4817-10-F

We seek a marine biologist with a proven record of world-leading research to join a dynamic group of biologists working in areas spanning marine ecology, molecular biology and marine biotechnology. The Centre offers well equipped laboratories including molecular biology, a research aquarium, high pressure lab equipment, a flow cytometer facility and scanning electron microscope plus access to sea going state-of-the-art sampling equipment including the remotely operated vehicle ISIS. We invite applications from individuals in any area of marine biology but with preferred emphasis on benthic or pelagic ecology of metazoa, fisheries biology, or marine biotechnology.

You will be expected to offer research leadership to the marine biology staff in the School and to foster close links with local NERC colleagues and staff in the Schools of Medicine and Biological Sciences through the new Institute for Life Sciences. You will be expected to contribute significantly to research-led education in marine biology within the School.

Chair in Physical Oceanography/ Ocean Remote Sensing

From £58,092 pa | Ref: 4812-10-F

Lectureship in Physical Oceanography/ Ocean Remote Sensing

£27,319 - £43,840 pa | Ref: 4818-10-F

We invite applications from individuals studying all areas of physical oceanography, ocean remote sensing and the role of the oceans in climate, including both model-based and observational studies and ranging from the coastal zone to the deep ocean. The Centre enjoys a rich heritage in physical oceanography, bringing together around 60 - 70 staff and research students. Ongoing research encompass major projects to study and monitor the Atlantic meridional overturning circulation, from the Southern Ocean to the Arctic, ground-breaking experiments to investigate mixing and sub-mesoscale processes in the Southern Ocean, and pioneering model studies of ocean dynamics and the ocean's role in the wider Earth system. These projects and activities help to underpin a strong portfolio of research-led education, to which you will be expected to contribute.

You must have a PhD at the time of appointment and a proven ability to publish innovative, high quality research. Emphasis will be placed on your track record of publications and grant capture commensurate to career stage and your ability to develop a dynamic, externally funded research program with an international profile. We welcome your application if you pursue fundamental research or if you have the potential to develop applied research and closer links with industry. You will have a commitment to excellence in undergraduate education, including field training in marine biology, oceanography or earth science and a desire to engage in doctoral student supervision by capitalizing on the opportunities presented by our large Graduate School. Details of our degree programmes can be found at http://www.southampton.ac.uk/soes/

Enquiries should be directed to Professor Tim Minshull, Head of the School of Ocean and Earth Science, email: tmin@noc.soton.ac.uk

To apply online visit www.jobs.soton.ac.uk Alternatively telephone 023 8059 2750. Please quote the relevant reference number (as above) on all correspondence. The closing date for all positions is 20 November 2010 at 12 noon. Our online system will prompt you to submit your curriculum vitae, concise statements of research interests and aspirations and teaching philosophy and interests, and the names and contact details of at least three referees. Please also send an electronic copy to Professor Minshull.

At the University of Southampton we promote equality and value diversity.

www.jobs.soton.ac.uk

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UNIVERSITY OF Southampton

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TENURE-TRACK FACULTY POSITION

MEDICAL COLLEGE OF WISCONSIN

DEPARTMENT OF BIOCHEMISTRY

The Department of Biochemistry invites applications for a tenure-track at the **Assistant or Associate Professor** level. We are seeking individuals to establish internationally leading programs that apply the tools of structural biology to topics of biomedical relevance. The Medical College of Wisconsin (**www.mcw.edu**) is the largest private research institution in Wisconsin, conducting over $142 million annually in funded research, and the Department of Biochemistry is home to state-of-the-art X-ray and NMR facilities. Institutional disease-focused initiatives provide translational research opportunities for candidates with an interest in structure-based drug discovery in many areas including cancer, cardiovascular disease and diabetes. Applicants must have a doctoral degree, at least 2 years of postdoctoral training, a strong publication record, and potential to obtain extramural funding. Competitive salary support, start-up funds and new laboratory space will be provided.

Applications should include a cover letter, curriculum vitae, statement of research interests, copies of key publications, and three reference letters. Send application materials and reference letters electronically in pdf format to:

Structural Biology Search Committee
Department of Biochemistry
Medical College of Wisconsin
8701 Watertown Plank Road
Milwaukee, WI 53226
E-mail: akear@mcw.edu

EOE M/F/D/V **(www.mcw.edu/hr)**

Invest your career where you can
Make a Difference!

Assistant Professor

Duke University School of Medicine

Applications are invited for a tenure track appointment as Assistant Professor in the Department of Pharmacology and Cancer Biology in the Duke University School of Medicine. The department faculty members have diverse interests that include cell signaling and regulation, cancer biology, gene regulation, chemical biology, neuropharmacology and metabolism.

Applicants should submit curriculum vitae, a brief statement of past research accomplishments and future research interests and a list of three references as a single PDF and should arrange for three letters of recommendation to be sent by email to the Pharmacology and Cancer Biology Search Committee 2010 at: **PCBSearch@mc.duke.edu**

Applications and letters are due by December 1, 2010.

Duke University
School of Medicine

Duke University Medical Center is an equal opportunity/affirmative action employer.

The Gatsby Charitable Foundation

UNIVERSITY OF CAMBRIDGE

Research Group Leader, Sainsbury Laboratory, Cambridge University

The Gatsby Charitable Foundation invites applications for Research Group Leader positions to be held at the Sainsbury Laboratory at the University of Cambridge.

The positions are open to applicants of any nationality. The Gatsby Charitable Foundation intends that the positions will be awarded across a broad range of seniority, but applicants would typically have postdoctoral experience in a field related to the overall scientific focus of the Laboratory in plant development and in computational modelling of development. Applicants should be able to provide strong evidence of their potential to become independent scientific group leaders. Applicants who currently hold a permanent post in a University or other Research Organisation are welcome to apply.

Funding will initially be provided for five years, with a possible extension for a further five year term, with no overall limit on number of extensions. The laboratory has excellent core facilities, including growth room, glasshouse and computing facilities, Group Leaders will be provided with an annual research grant exceeding £250,000 per annum in addition to their salary. Once in post, group leaders will be eligible to apply for research grant funding from other sources.

Applications should be submitted electronically as PDF files and include a full CV, a research proposal for the initial five year period as group leader, and the names of at least three referees. Further guidance and details about the laboratory can be obtained by e-mailing **Sainsburylaboratory@admin.cam.ac.uk**. Completed applications should be sent by e-mail to **Sainsbury.applications@admin.cam.ac.uk**.

Applications can be submitted at any time. Applications will be peer reviewed by the Gatsby Charitable Foundation at least three times annually.

THE UNIVERSITY OF ARIZONA

DEPARTMENT OF IMMUNOBIOLOGY

Applicants are invited for Tenured/Tenure Track positions at the assistant, associate, and full professor rank, depending upon qualifications, at the, Department of Immunobiology, University of Arizona College of Medicine. Qualified applicants will develop strong, extramurally funded programs. Preference will be given to those candidates investigating initiation and regulation of innate and inflammatory responses, however, applicants with outstanding programs in other areas of immunobiology and microbial or immune pathogenesis will be considered as well. Successful applicants are expected to not only develop independent research programs and contribute to graduate (Ph.D.) and medical (M.D.) education, but also to invest a fraction of their effort to help build interactive and collaborative programs within and outside the Department to tackle larger biomedical problems relevant to human health.

The University of Arizona is amongst the top 20 public research and education universities, boasting excellent core facilities, strong departments and centers, as well as lively campus culture and life, and a blossoming recreations center. It is located in sunny Tucson, a city with a vibrant multicultural population of approximately 900,000, strong economy, and business. Tucson is surrounded by a majestic desert and mountains rising to more than 9,000 feet.

Please complete an online application for **Job #44945** at **www.hr.arizona.edu**. Be prepared to attach your curriculum vitae.

The University of Arizona is an EEO/AA Employer – M/W/D/V.

Founded in 1911, The University of Hong Kong is committed to the highest international standards of excellence in teaching and research, and has been at the international forefront of academic scholarship for many years. Ranked 24th among the top 200 universities in the world by the UK's *Times Higher Education*, the University has a comprehensive range of study programmes and research disciplines spread across 10 faculties and about 100 sub-divisions of studies and learning. There are over 23,400 undergraduate and postgraduate students coming from 50 countries, and more than 1,200 members of academic and academic-related staff, many of whom are internationally renowned.

Post-doctoral Fellowships and Research Assistant Professorships

Applications are invited for a number of positions as Post-doctoral Fellow (PDF) (Ref: 20100576) and Research Assistant Professor (RAP) (Ref: 20100577), at the University of Hong Kong, on or before July 31, 2011. Appointments will be made for a period of 2 to 3 years.

PDF and RAP posts are created specifically to bring new impetus and vigour to the University's research enterprise. Positions are available from time to time to meet the strategic research needs identified by the University. Positions are available in the following Departments:

- Real Estate and Construction
- School of Humanities (History)
- Faculty of Dentistry
- Faculty of Education
- Institute of Human Performance
- Electrical and Electronic Engineering
- Mechanical Engineering
- Biochemistry
- Centre for Cancer Research
- Clinical Oncology
- Research Centre of Infection and Immunology
- Medicine
- Physiology
- Psychiatry
- Centre for Reproduction, Development and Growth
- Chemistry
- Earth Sciences
- Geography
- HKJC Centre for Suicide Research and Prevention
- The State Key Laboratory of Brain and Cognitive Sciences
- Area of Excellence (AoE) on The Institute of Molecular Technology for Drug Discovery and Synthesis

Post-doctoral Fellows

PDFs are expected to devote full-time to research. Applicants should be doctoral degree holders having undertaken original research that has contributed to the body of knowledge. A highly competitive salary commensurate with qualifications and experience will be offered. Annual leave and medical benefits will also be available.

Research Assistant Professors

The main focus of an RAP's duty is research. RAPs can however be assigned some teaching duties, up to 50% of the normal teaching load. Applicants should be research active and have a proven publication record. A highly competitive salary commensurate with qualifications and experience will be offered, with a contract-end gratuity and University contribution to a retirement benefits scheme (totalling up to 15% of basic salary). Annual leave, and medical/dental benefits will also be offered.

Procedures

Prospective applicants are invited to visit the following webpage <http:// www.hku.hk/apptunit/> to view the full list of the research areas and their home Faculties/Departments/Schools/Centres for which PDF/RAP positions are currently available. Before preparing an application they should contact the Head of the appropriate academic unit to ascertain that their research expertise matches the research area for which a vacant PDF/RAP post is available.

Applicants must submit a completed University application form, which should clearly state **which position** they are applying for; and in which **academic discipline**. They should also provide further information such as details of their research experience, publications, research proposals, etc.

Further particulars and application forms (152/708) can be obtained at http://www.hku.hk/apptunit/; or from the Appointments Unit (Senior), Human Resource Section, Registry, The University of Hong Kong, Hong Kong (**fax**: (852) 2540 6735 or 2559 2058; **e-mail**: senrappt@hku.hk). **Closes October 16, 2010**. Candidates who are not contacted within 3 months of the closing date may consider their applications unsuccessful.

The University is an equal opportunity employer and is committed to a No-Smoking Policy

Scientist Positions at Novo Nordisk China R&D Center

Zhongguancun Life Science Park, Beijing, China

Novo Nordisk is a world leading healthcare company in diabetes care, haemophilia treatment, growth hormone therapy and hormone replacement therapy. Novo Nordisk China R&D Center is an integrated part of Novo Nordisk R&D organization and now looking for highly motivated scientists who plan to advance their career in China. For all positions, excellent communication skills in spoken and written English, flexibility and the desire to work within a multidisciplinary team are required. Industry experience is a preferred. Title and compensation will be based on qualifications. For application, please send resume and cover letter **with job code in the subject of email to: InfoNNST@novonordisk.com**.

- Scientist/Senior Scientist, Antibody Phage Display [Job Code: MB_201009_Phage]:

Responsibility:

- Lead effort on antibody discovery/generation via phage display selection process
- Contribute to strategic and scientific activities related to the discovery and design of recombinant antibody
- Interact with discovery scientists and develop effective inter-departmental and cross-functional relationships, including international interactions
- Identify, evaluate and manage potential external collaborators and research opportunities
- Expand awareness of novel technology development for antibody & protein therapeutics

Requirements:

- Ph.D. in Molecular Biology, Biochemistry or Immunology, and a minimum of 3 years of relevant post-doc experience
- A proven track record with phage display or other display platforms
- Solid knowledge and hands-on in-depth experience in various molecular biology techniques, particularly in antibody library construction, engineering, expression, purification and characterization.
- Experience with automation and high throughput screening is a plus.

- Scientist/Senior Scientist, Fermentation technology [Job Code: MB_201009_Fermentation]:

Responsibility:

Plan and execute laboratory- and mini-pilot-scale E coli cultivations to support the development of Novo Nordisk biologics pipeline. In this role you are expected to design experimental programs, develop cultivation protocols and oversee the execution.

Requirements:

- Ph.D. in Biochemical/Chemical Engineering w/good track record of research in fermentation or bioprocess development.
- In-depth knowledge of microbial physiology and metabolism, fermentation technology and recovery processes.
- Competent with basic analytical tools/laboratory automation – HPLC, microscopy, plate readers, automated liquid handling, high throughput micro culture systems and fermentor automation and control.
- Experience in a biotech or pharmaceutical environment and knowledge of statistical data analysis methodology is preferred.

- Scientist/Senior Scientist in Cell Biology [Job Code: CB_201009_Sci]:

Responsibility:

Using immunology, cell biology, and antibody technology expertise to actively participate in and contribute to the novel drug R&D for the treatment of inflammatory disorders.

Requirements:

- Ph.D. in Immunology or Cell Biology with excellent track record. •>2 years of postdoctoral experience in the disciplines mentioned above.
- Rich experience in cell based functional assays and using various FACS equipments
- Experience in inflammatory disease research or monoclonal antibody technology development is preferred.

Furthermore, we also have 2 positions within diabetes research department:

- Senior Scientist in protein purification with experience in process development [Job Code: DR_201009_PPPD]

- Scientist in molecular biology/ protein expression with special focus on E.coli [Job Code: DR_201009_MB/PE]

For full description of requirements, please visit: **http://www.ebiotrade.com/jobf/readall1.asp?cmpidn=y2003527103359**.

Science Careers online @sciencecareers.org

UNIVERSITY OF UTAH SCHOOL OF MEDICINE

Tenure-track Assistant and/or Associate Professor of Human Genetics

The Department of Human Genetics in the University of Utah School of Medicine (**http://genetics.utah.edu/**) is seeking outstanding applicants for a tenure-track position at the level of Assistant or Associate Professor. We encourage applications from scientists with interests in genetic approaches to complex disease, population/statistical genetics, computational biology, functional genomics, and animal models of human disease. Our department has a strong history in human genetics, genomics, and developmental genetics, and our faculty members collaborate closely with faculty in other basic science and clinical departments. Creative scientists with a record of achievement and commitment to excellence in both research and teaching are encouraged to apply. Successful candidates will receive a highly competitive startup package and enjoy a stimulating and supportive research environment.

Applicants should submit an electronic version of current CV, summary of research plans, relevant reprints and/or preprints, and three letters of reference to:

Dr. Lynn B. Jorde
Professor and Chair
c/o Natalie Johnson, njohnson@genetics.utah.edu

Application materials, including letters of reference, must be submitted by **November 1, 2010**.

The University of Utah is an Affirmative Action/Equal Opportunity Employer and does not discriminate based upon race, national origin, color, religion, sex, age, sexual orientation, gender identity/expression, disability, or status as a Protected Veteran. Upon request, reasonable accommodations in the application process will be provided to individuals with disabilities. To inquire about the University's nondiscrimination policy or to request disability accommodation, please contact: Director, Office of Equal Opportunity and Affirmative Action, 201 S. Presidents Circle, Rm 135, (801)581-8365. The University of Utah values candidates who have experience working in settings with students from diverse backgrounds, and possess a demonstrated commitment to improving access to higher education for historically underrepresented students.

FLORIDA GULF COAST UNIVERSITY

In response to continued enrollment growth FGCU announces the search for highly qualified applicants to fill the following positions:

College of Arts & Sciences

Biological Sciences, Associate/Full Professor, Req. #1233
(Application Deadline: December 1, 2010)

Biology, Instructor I, Req. #1263 **(Application Deadline: December 2, 2010)**

Hydrodynamic Modeler, Assistant/Associate Professor, Req. #1232
(Application Deadline: November 5, 2010)

Sustainability Science, Associate Professor, Req. #1216
(Application Deadline: November 6, 2010)

Paleoclimatology, Assistant/Associate Professor, Req. #1260
(Application Deadline: November 5, 2010)

Sproul Chair, Habitat Restoration & Management, Eminent Scholar, Req. #1230 **(Position is open until filled, however; for first consideration, apply before November 15, 2010.)**

To apply for any of these positions, please visit our website at **http://jobs.fgcu.edu** and access the Req. # for detailed information. **Application materials will only be accepted online.** All application materials must be received by the deadline date of the position. ***Application packages, including additional materials submitted such as videos, tapes, slides, books, etc., are subject to public review under Florida's Public Records law, shall become the property of FGCU, and cannot be returned.*** Finalists will be required to provide official transcripts.

FGCU is an EOE, which has a commitment to cultural, racial, and ethnic communities and encourages women and minorities to apply. It is expected that successful candidates share this commitment.

THE UNIVERSITY OF CHICAGO

Virology Faculty Position
Req # 00564

The Department of Microbiology at The University of Chicago invites applications for a position at the rank of Assistant, Associate or full Professor. The successful applicant is expected to develop a research program that complements the interests of the current faculty on pathogen-host interactions. Candidates are expected to develop an extramurally funded research program and contribute to departmental teaching. The University of Chicago maintains extensive core facilities in support of research including specialized facilities for human pathogen research.

Applicants for the position of Assistant or Associate Professor should have a productive research experience with first author publications in virology during postdoctoral training. Applicants for senior positions should have a a sustained, vigorous, externally funded research program in virology. Competitive salaries and start-up packages will be provided. Applicants must submit a cover letter, curriculum vitae and a statement of research interests emphasizing career goals at The University of Chicago's Academic Careers Opportunities website, **http://tinyurl.com/2vjyauh**. Applicants to the Assistant Professor position must arrange to have 3 letters of recommendation submitted to **Melissa Harmeyer** (**mharmeye@bsd.uchicago.edu**). The deadline for submission of all application materials is **November 15, 2010**.

The University of Chicago is an Affirmative Action / Equal Opportunity Employer.

http://tinyurl.com/2vjyauh

Washington University in St. Louis
SCHOOL OF MEDICINE

Tenure Track Faculty Positions in Developmental/Regenerative Biology

The Department of Developmental Biology at Washington University School of Medicine is undergoing a major expansion and is recruiting outstanding scientists interested in developing innovative, independent research programs focused on stem cell or regenerative biology. The research interests of the present faculty in the Department of Developmental Biology are diverse and exploit a variety of model organisms, including C. elegans, D. melanogaster, Xenopus, zebrafish, and mouse (**http://DevBio.wustl.edu**). Applications will be accepted from individuals holding the Ph.D., M.D., or M.D./Ph.D. degrees and having relevant postdoctoral training. Appointments will be made at the level of Assistant or Associate Professor on the tenure track.

Interested individuals should send a letter of interest, curriculum vitae and summary of research accomplishments and plans (in a single PDF file) by **November 1, 2010** to:

Search Committee (c/o Lilianna Solnica-Krezel, Chair)
Department of Developmental Biology
Washington University School of Medicine
660 South Euclid Avenue, Campus Box 8103
St. Louis, MO 63110
Email: devbiosearch@wustl.edu

Applicants should also arrange to have three letters of recommendation (pdf files) sent to **devbiosearch@wustl.edu**.

Washington University is an Equal Opportunity Employer AA/EOE M/F/D/V.

University at Buffalo
The State University of New York

Chair, Department of Microbiology and Immunology

The School of Medicine and Biomedical Sciences at The State University of New York at Buffalo (University at Buffalo; UB), invites nominations and applications for the position of Professor and Chair of the Department of Microbiology and Immunology. The new Chair will be expected to provide the scientific vision and direction to bring the Department to the next level of excellence. Appropriate resources, in the form of new Assistant to Full Professor faculty lines and startup packages, are available to implement this growth.

DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY: Currently, there are 16 faculty members with interest in bacteriology, virology, parasitology, and immunology, and, most recently, mycology. Current faculty is collegial, committed, and ambitious and active in all aspects of academia, with a total direct cost grant portfolios over $20M. The Department (**http://www.smbs.buffalo.edu/microb/Academic_Programs/**) is responsible for educating students in both Medical and Dental school and health related professions, and for training of its own graduate and undergraduate students . The Department plays a major role in the Witebsky Center for Microbial Pathogenesis and Immunology, where faculty from several Departments works in contiguous space.

The University at Buffalo is entering the fifth year of implementation of the UB2020 strategic plan, with major strengths in Molecular Recognition in Biological Systems, Bioinformatics, and Health and Wellness across the Lifespan. UB is the SUNY system's comprehensive campus; the Health Sciences complex includes the Schools of Dental Medicine, Pharmacy, Public Health and Health Professions, and Nursing in addition to Medicine and Biomedical Sciences.

STATE-OF-THE-ART FACILITIES: Modern and well-designed laboratory space, as well as cutting edge research cores in many areas, including genomics, proteomics, microscopy/imaging, macromolecular crystallization, and transgenic animals are available. Diverse opportunities for collaborations exist at nearby Roswell Park Cancer Institute, the Hauptman-Woodward Medical Research Institute, the New York Center of Excellence for Bioinformatics and Life Sciences, the Research Institute on Addictions, and the Center for Excellence in PK/PD in the Department of Pharmaceutical Sciences.

COMMUNITY ENVIRONMENT: Buffalo has unique characteristics, which make it an ideal place to live and work. Buffalo, called "The City of Good Neighbors", is the second largest city in New York State. Amherst, a nearby suburb, is consistently rated as one of the safest communities in the nation. Area public schools are outstanding and housing is very affordable. Buffalo is a cultural center with excellent theater, museums, and an exceptional symphony. In addition, the surrounding environment contains two of the Great Lakes for sailing, boating and fishing, hills for skiing and hiking and the beauty of Niagara Falls which combine to create an optimal environment. To learn more about the Buffalo/Niagara region visit: **www.buffalo.edu/aboutub/region.html**.

CANDIDATE REQUIREMENTS: The successful candidate will have a Ph.D., M.D. or equivalent degree, and a well-funded and internationally recognized program of research in the broadly defined area of Microbiology and Immunology. Strong leadership and administrative skills are essential attributes. Beyond maintaining a strong research program, responsibilities of the Chair include administration of the Department and its teaching programs, as well as defining its scientific vision and overall directions.

APPLICATION PROCESS: Applications should be addressed to Dr. M. L. Dubocovich, Chair, Microbiology and Immunology Search Committee, and submitted to **www.ubjobs.buffalo.edu** (posting # 1000474). Nominations or inquiries may be sent to **mdubo@buffalo.edu**. Position will be open until filled.

The University at Buffalo is an Equal Opportunity/Affirmative Action Employer/Recruiter.

University of Massachusetts
UMASS Medical School

Science Director
Worcester, MA

The Program in Molecular Medicine at the University of Massachusetts Medical School seeks a Science Director to identify and facilitate opportunities for collaborative basic and translational research funding, including trainee funding. With the Chair and Principal Investigators, the Science Director facilitates the preparation, writing and submission of applications to federal, private, foundations, and industry agencies for funding exciting collaborative research initiatives of outstanding faculty teams. Major responsibilities also include determining which opportunities are feasible and appropriate; facilitating implementation of project plans to attain specific milestones and leverage resources; and directing efforts to set project priorities, track progress, obtain agreements with staff, and ensure successful implementation.

Candidates must possess: a doctorate in the life sciences and 3 years of experience in a project management role in business or academic environments or a life science editorial position, with experience in publications and grant funding; understanding of national trends affecting clinical and translational research; familiarity with the policies and procedures of federal and other sponsors of clinical and translational research; successful grant-writing of NIH institutional grant applications.

The Program in Molecular Medicine is comprised of 39 faculty laboratories with highly successful, funded research programs, including 3 HHMI investigators. We offer highly competitive salaries, state retirement, generous time off policies, 13 paid holidays, paid continuing education, health, dental and vision coverage.

Apply online by visiting our website at www.umassmed.edu; Human Resources, Open Positions, Search for position # 18786.

As an equal opportunity and affirmative action employer, UMMS recognizes the power of a diverse community and encourages applications from individuals with varied experiences, perspectives and backgrounds.

University of Michigan

Center for Computational Medicine and Bioinformatics
Assistant, Associate and Full Professors

The Center for Computational Medicine and Bioinformatics (CCMB) of the University of Michigan seeks outstanding applicants for tenure-track and tenured bioinformatics and biomedical informatics faculty positions at all levels. CCMB and its Bioinformatics Graduate Program (BGP) facilitate interdisciplinary research and education, and forge collaborative relationships with faculty and programs across the Medical School, the University, and nationally. CCMB houses the NIH-funded National Center for Integrative Biomedical Informatics (NCIBI.org), the UM Clinical and Translational Sciences Award (CTSA) Biomedical Informatics Program, 48 current Bioinformatics PhD students, and NIGMS and NCI bioinformatics and proteome informatics training grants. We have active national outreach for minority candidates.

CCMB is currently recruiting up to 3 senior and 5 junior faculty to establish independent individual and team-based research programs. There are opportunities to lead components of CCMB programs. Informatics research teams spanning parts of the basic to clinical spectrum are welcome to apply as a unit. Specific partnerships are available with the Dept. of Human Genetics, the Comprehensive Cancer Center, the Systems Biology Division of the Dept. of Molecular & Integrative Physiology, the Brehm Center for Type 1 Diabetes Research and Analysis, and the Michigan Institute for Clinical and Health Research. For appropriate individuals, faculty leadership roles in Research Information Technology management and operations are feasible more broadly in the Medical School and UM Health System. There are extensive computational and information infrastructural resources available to CCMB faculty and programs.

Successful candidates will have a PhD and/or MD degree and may have postdoctoral training in genomic; epigenomic, transcriptomic, proteomic, metabolomic or metagenomic, discovery and analysis; computational biology and modeling; biomedical data mining and multi-scale integrative analysis; natural language processing (NLP) and ontologies applied to biomedicine; or informatics related to healthcare delivery and personalized medicine. Publications and grants as evidence of research productivity and interest in active participation in the CCMB graduate and postdoctoral instructional programs are essential. The rank of selected candidates will depend upon experience and qualifications.

Applicants should send a letter of interest with Curriculum Vitae, Research Plan, and a list of three or more references to: **Search Committee, Center for Computational Medicine and Bioinformatics, Job Code 200. The University of Michigan, 2017 Palmer Commons, 100 Washtenaw Ave, Ann Arbor, MI. 48109-2218**, email: **ccmbrecruit@umich.edu**. For appropriately qualified candidates, simultaneous application to the UM Biological Sciences Scholars Program, BSSP, is strongly encouraged (for more information see: **http://www.med.umich.edu/medschool/research/bssp/**). Applications will be reviewed through January.

The University of Michigan is an Equal Opportunity/Affirmative Action Employer.

POSITIONS OPEN

Jacob's Cure is proud to announce the **STARKER FELLOWSHIP** for White Matter Disease Clinical Research, a two-year Clinical Research Training Fellowship to support research into the cause, treatment, or cure of white matter or neurodegenerative disease. The award will consist of a commitment of $49,500 per year for two years to support salary and/or research costs. For eligibility requirements and application instructions, please visit **website: http://www.jacobscure.org/fellowship** or **e-mail sarah@jacobscure.org.**

Central Michigan University (CMU) seeks a dynamic and creative electrochemist for a tenure-track **ASSISTANT PROFESSOR** position in the Department of Chemistry and the interdisciplinary Science of Advanced Materials (SAM) Ph.D. program to begin August 2011. This position adds to a newly formed group exploring new materials for alternative and renewable energy applications. The preferred candidate will develop a competitive, externally funded research program in electrochemistry with additional emphasis on the synthesis and characterization of inorganic-based materials or nanomaterials. In addition, it is expected that the candidate will participate in the SAM Ph.D. program, and successfully teach introductory, undergraduate, and graduate courses in one or more of the traditional sub-disciplines of chemistry and in the area of specialization. The position is structured such that teaching activities will begin during the third year of appointment, with the establishment of a competitive research program serving as the major focus during the first two years of the appointment. Additional responsibilities include advising, supervising student research, and department and university service. Classified by the Carnegie Foundation as a doctoral research university, CMU is recognized for strong undergraduate education and a range of focused graduate and research programs. The department offers bachelor's degrees in chemistry, biochemistry, chemistry education, and master's degrees in chemistry and chemistry education. The new, interdisciplinary Ph.D. program in the Science of Advanced Materials was initiated in fall 2008 (**website: http://www.sam.cmich.edu**). Ph.D. in materials-based Inorganic Chemistry, Materials Science, or closely related chemistry discipline with electrochemical focus, demonstrated effective communication skills, and demonstrated potential for excellence in teaching and research required. Postdoctoral research experience in materials for energy applications strongly preferred. Application materials to include vitae, unofficial transcripts, proposed research agenda, description of equipment and startup needs, and statement of teaching philosophy should be submitted at **website: http://www.jobs.cmich.edu.** The applicant should arrange for three professional letters of reference to be sent separately to: **Chair, Search Committee, Department of Chemistry, Central Michigan University, Mount Pleasant, MI 48859-0001. Telephone: 989-774-3981. Fax: 989-774-3883. E-mail: chem@cmich.edu.** Review of materials will begin on October 15, 2010, and will continue until position is filled. Further information on the Department of Chemistry may be viewed at **website: http://www.chm.cmich.edu/.** *CMU, an Affirmative Action/Equal Opportunity Institution, strongly and actively strives to increase diversity within its community (see* **website: http://www.cmich.edu/aaeo/**).

Translational microbiology and microbial pathogenesis science. The University of Arizona seeks to hire a tenure-eligible physician-scientist at the **ASSISTANT** or **ASSOCIATE PROFESSOR** level in Infectious Disease. M.D.-Ph.D.s are particularly encouraged to apply. To apply, visit **website: http://www.uacareertrack.com,** job number **45225,** or contact search committee chair, **Magdelene So, Ph.D.** at **e-mail: somaggie@e-mail.arizona.edu.**

POSITIONS OPEN

Duke Children's HOSPITAL & HEALTH CENTER

The Department of Pediatrics and the Department of Immunology at Duke University Medical Center seek an established **PHYSICIAN SCIENTIST** who has a track record of success in the field of basic immunology with an emphasis on primary immunodeficiency and with potential for translational research. This individual will expand existing strengths in basic and clinical immunology at Duke and will contribute to graduate education. There will be additional opportunities to contribute to the Program in Microbial Pathogenesis, Emerging Infections, and Hosts Defense and to broaden collaborations with the Institute for Genome Sciences and Policy, the Duke Translational Medicine Institute, the Duke Human Vaccine Institute, the Department of Molecular Genetics and Microbiology, and other groups across the Duke University campus.

Interested individuals should send a letter of application, curriculum vitae, and list of references to either **Wesley Burks, M.D.,** chief of the Division of Pediatric Allergy-Immunology, at **e-mail: wesley.burks@duke.edu** or **Michael Krangel, Ph.D.,** interim chair of the Department of Immunology, at **e-mail: krang001@mc.duke.**

Duke University Health System is an Equal Opportunity/Affirmative Action Employer.

ASSISTANT, ASSOCIATE, or FULL PROFESSOR

The James Graham Brown Cancer Center at the University of Louisville seeks two scientists at the Assistant/Associate/Full Professor level to participate in the Kosair Charities Pediatric Cancer Research Program (KCRP). This new program will focus on the discovery and development of new anticancer targets and drugs for pediatric malignancies. KCRP is funded by a $12 million commitment from the Kosair Charities Foundation (Louisville, Kentucky) which will provide support for faculty recruitment and support, research infrastructure, and clinical trials. The participants of the KCRP will have or will develop competitive research programs and will be faculty members at the James Graham Brown Cancer Center, University of Louisville. KCRP will interface with the very strong translational program of the Brown Cancer Center. The Brown Cancer Center drug development program comprises more than 40 scientists and has had considerable experience with novel drug development. It has developed three drugs that are in early phase clinical trials, with more than twenty novel compounds in preclinical development. The Brown Cancer Center has a complete spectrum of shared facilities to enhance target characterization and drug identification.

Please send letters of application and curriculum vitae to:

John O. Trent, Ph.D.
Director, KCRP, James Graham Brown Cancer Center
505 South Hancock Street
Louisville, KY 40202
E-mail: john.trent@louisville.edu.

FACULTY POSITION in IMMUNOLOGY
Boston University School of Medicine

The Department of Microbiology (**website: http://www.bumc.bu.edu/microbiology**) is seeking outstanding investigators with an innovative record in research for immunology faculty position(s). Applications in all areas of immunology will be considered, but investigators specializing in basic aspects of adaptive immunity, the immunology of infectious diseases, and/or the use of computational approaches to understanding the immune system or for vaccine design are especially encouraged to apply. Candidates appropriate for any faculty level position will be considered.

Interested individuals should submit their curriculum vitae, summary of research accomplishments, future research plans, and the names of at least three references to **e-mail: kfurness@bu.edu** no later than December 1, 2010.

Boston University School of Medicine is an Equal Opportunity/Affirmative Action Employer.

POSITIONS OPEN

FACULTY OPPORTUNITY
Assistant/Associate Professor of Microbiology

Mount Sinai School of Medicine is a leader in medical research and education. The Department of Microbiology is significantly expanding its research program in the area of virus-host interactions. We are currently seeking an individual who will develop a well-funded, active, independent research program. The successful candidate will join a multidisciplinary department focusing on molecular and genetic aspects of infectious diseases, host-pathogen interactions, and immunological host responses to infection. They will also participate in teaching at the graduate and medical school levels.

For consideration, please send your curriculum vitae, selected reprints, names and addresses of three references, and a statement of research interest to:

Chair, Search Committee
Department of Microbiology, Box 1124
Mount Sinai School of Medicine
One Gustave L. Levy Place
New York, NY 10029-6574

You may also send to **e-mail: nina.umerah@mssm.edu.** Please visit the department online at our **website: http://www.mssm.edu/departments-and-institutes/microbiology.**

We are an Equal Opportunity Employer fostering diversity in the workplace.

COGNITIVE NEUROSCIENCE

As part of a major research growth initiative, The University of Wisconsin-Milwaukee is seeking applicants for a faculty position in Cognitive Neurosciences. This position will build directly on the current strength of our program (**website: http://neuroscience.uwm.edu**) and the appointment will be made at the **ASSISTANT PROFESSOR** level.

Successful applicants will participate in the Center for Imaging Research (**website: http://www.firc.mcw.edu**) which provides multiple research dedicated imaging platforms and technical infrastructure for brain mapping in human subjects and laboratory animals. Area of research specialization is open but outstanding individuals with interests in attention, perception, action, language, or emotion are particularly encouraged to apply. Responsibilities include developing an independent extramurally funded research program and teaching graduate and undergraduate courses in the neurosciences and experimental psychology.

Applicants must have a Ph.D. in neuroscience or a closely related field, research interests in cognitive neuroscience, and significant postdoctoral research experience. Preferred qualifications are strong potential for extramural funding; demonstrated research productivity; interest in fostering graduate and undergraduate research experience; excellence in graduate and undergraduate teaching; and the ability to strengthen or expand existing programs.

Review of applications will begin on November 15, 2010, and continue until the positions are filled. To apply online please see **website: http://jobs.uwm.edu/postings/4938**

A complete application will consist of a cover letter, vita, a concise statement of research interests, and three letters of reference. Candidates may submit a statement of teaching interests. All application materials may be submitted electronically, except applicants should arrange for three letters of reference to be mailed to: **Neuroscience Search Committee, Department of Psychology, UWM, PO Box 413, Milwaukee, WI 53201.** *UWM is an Equal Opportunity Institution committed to diversity.*

THE UNIVERSITY OF CHICAGO

Faculty Openings In Neurobiology

The Department of Neurobiology at the University of Chicago seeks to recruit tenure-track faculty. Appointments can be made at any level, and applicants in any area of neurobiology are encouraged with a preference for individuals investigating synaptic transmission/plasticity or neural development. Candidates must have a PhD and at least two years of postdoctoral training in Biological Sciences.

Applicants should apply online at **academiccareers.uchicago.edu/applicants/Central?quickFind=51283** by uploading a cover letter, CV, a statement of research objectives, and the names and contact information of three academic references. Applicants must arrange to have 3 letters of recommendation submitted to **Elizabeth Grove c/o NeuroSearch@bsd.uchicago.edu**. Applications will be accepted until the position(s) are filled, application review will begin **November 1, 2010**.

The University of Chicago is an Affirmative Action/Equal Opportunity Employer.

UNIVERSITY of DAYTON

THE DEPARTMENT OF CHEMISTRY AT THE UNIVERSITY OF DAYTON invites applications for a tenure-track faculty position in fields related to **Biochemistry** (biochemistry, pharmacology, toxicology, etc.) at the rank of **Assistant Professor** starting Fall 2011. The Chemistry Department offers an ACS approved B.S. degree in Chemistry, a B.S. degree in Biochemistry, a B.A. degree in Chemistry and a M.S. degree in Chemistry. We seek an individual who will thrive in an environment that combines excellence in both teaching and research.

Candidates must have a strong interest in teaching, strong communication skills and an expressed interest in academic and research advising of undergraduate Chemistry and Biochemistry majors. The successful candidate will be expected to teach courses in biochemistry, their area of specialization, and introductory chemistry courses, and to establish an innovative, externally funded research program including both undergraduates and M.S. students. A Ph.D. in a biochemically related field and at least one year of postdoctoral research experience are required. Candidates with experience working with diverse student populations and who can contribute to proposed medicinal/pharmaceutical chemistry B.S. and existing bioengineering M.S. programs are preferred. The University of Dayton has received a National Science Foundation ADVANCE grant designed to promote the advancement and representation of women in the sciences.

To apply, submit a *curriculum vitae*, descriptions of teaching philosophy and research plans including equipment and startup needs, and copies of undergraduate and graduate transcripts to **http://jobs.udayton.edu/applicants/Central?quickFind=51402**. Three original letters of recommendation, at least one of which must address teaching potential, should be sent to: **Search Committee, Department of Chemistry, University of Dayton, Dayton, OH 45469-2357**. For additional information, or if you have questions, contact Dr. David Johnson at **dave.johnson@notes.udayton.edu**. All application materials must be received by November 1, 2010.

For more information about the department, please visit **www.udayton.edu/~chem**. The University of Dayton, a comprehensive Catholic University founded by the Society of Mary (the Marianists) in 1850, is Ohio's largest independent university and one of the nation's ten largest Catholic universities.

The University of Dayton is firmly committed to the principle of diversity and is an Affirmative Action/Equal Opportunity Employer. Persons of color, women, individuals with disabilities and veterans are encouraged to apply.

Director,

James H. Oliver, Jr., Institute of Arthropodology and Parasitology
Georgia Southern University

Georgia Southern University invites nominations and applications for the Director of the Institute of Arthropodology and Parasitology (IAP) at the associate or full professor rank. The full text announcement with all qualifications and application instructions is available at **http://www.bio.georgiasouthern.edu/searches**. Georgia Southern seeks to recruit individuals who are committed to excellence in administration, scholarship, teaching and professional service within the University and beyond and who are committed to working in diverse academic and professional communities.

The IAP serves as a center where persons with interests in parasitic arthropods and vector-borne diseases can interact and focus on research and applied problems. The U.S. National Tick Collection is an integral part of the IAP. It is the largest tick collection in the world with several million specimens representing approximately 90% of the world's species. The collection remains the property of the Smithsonian Institution, but is on long-term loan.

Screening of applications will begin **22 October 2010**. The position starting date is 1 August 2011. A complete application consists of a letter addressing the qualifications and duties cited in the full text announcement, a curriculum vitae, and letters from at least 3 professional references. Other documentation may be requested. Nominations, applications and letters of reference may be mailed as a hardcopy or in PDF format sent via e-mail.

Dr. Stephen Vives, Chair, Search #59318
Department of Biology
Georgia Southern University
P.O. Box 8042
Statesboro, GA 30460
e-mail: IAPDirectorSearch@georgiasouthern.edu
912-478-5487

Georgia Southern University is an AA/EO Institution and the state is an Open Records state. Finalists will be required to submit to a background investigation. Individuals who need reasonable accommodations under the ADA to participate in the search process should contact the Associate Provost.

Yale University School of Medicine
Interdepartmental CNNR Program
Cellular Neuroscience, Neurodegeneration, and Repair
PO Box 9812
New Haven, CT 06536-0812
http://medicine.yale.edu/cnnr/

Faculty Positions

The Yale Program in Cellular Neuroscience, Neurodegeneration, and Repair (CNNR) is searching for a scientist who uses **molecular and cellular** approaches to advance the understanding of **Nervous System** function. Both outstanding applicants with research programs focused on **understanding neurodegeneration or promoting neural repair**, and applicants with a focus on **basic aspects of neuronal function** are encouraged to apply. The successful applicant will receive a primary appointment in one of the departments of the Yale School of Medicine and will be active members of that department. Please see our website **http://medicine.yale.edu/cnnr**.

Candidates must hold an M.D. and/or a Ph.D. degree, or equivalent degrees. We invite applications at the rank of assistant professor, but appointments at the rank of associate professor will be considered. Applications are due by **November 15, 2010**. Please send a cover letter, curriculum vitae, up to 3 representative publications, a research plan (strictly limited to 2 pages), and arrange for submission of 3 letters of recommendation.

All application materials should be sent **electronically** to **Pietro De Camilli** and **Stephen M. Strittmatter**, directors of the Program, exclusively at the following e-mail address: **cnnr.search@yale.edu**.

Applications from women and minority scientists are encouraged. Yale is an Affirmative Action/Equal Opportunity Employer.

POSITIONS OPEN

ASSISTANT PROFESSOR - CLIMATE ECOLOGIST

The University of Oklahoma is seeking a new faculty member who works at the intersection of climate change and biological diversity to fill a 12-month, tenure-track position as an Assistant Professor jointly appointed between the Oklahoma Biological Survey and the Department of Botany and Microbiology, beginning 1 July 2011. We seek researchers who use modern tools to understand how changing climate patterns influence plant biodiversity and ecosystem function. The University of Oklahoma provides an ideal setting for addressing this question because of the sharp, east–west climate gradient across the southern Plains, the diversity of ecoregions and habitats, excellent infrastructure including world-class climate and genomics facilities, and strong faculty expertise in Meteorology, Climate, Molecular and Organismal Biology, and Ecology and Environmental Science. Responsibilities will include developing an externally funded research program and teaching one course per year in the candidate's area of expertise. A Ph.D. is required; postdoctoral experience is preferred. The successful applicant must demonstrate research productivity, the potential to attract external funding, and teaching skills. Screening will begin 15 December 2010 and continue until the position fills. Please send curriculum vitae, up to five sample reprints, and full contact information for four references to:

Michael A. Patten,
Search Committee Chair,
Oklahoma Biological Survey,
University of Oklahoma,
111 East Chesapeake Street, Norman,
Oklahoma 73019.

For additional information on programs at the Oklahoma Biological Survey and Department of Botany and Microbiology, please visit **websites: http://www.biosurvey.ou.edu/** and **http://www.ou.edu/cas/botany-micro/.** For information on the University's climate resources, see **website: http://www.ocs.ou.edu/.** *The University is an Equal Opportunity and Affirmative Action Employer. Woman and minorities are encouraged to apply.*

INTEGRATIVE BIOLOGY
University of North Dakota

We seek to fill a tenure-track position at the **ASSISTANT** or **ASSOCIATE** level with an individual conducting hypothesis driven research on the genomic basis of prokaryotic or eukaryotic adaptation to biotic and abiotic environmental factors and interactions.

The candidate's research should span multiple levels of biological organization and utilize cutting edge experimental approaches. The specific system is of less importance than is the paradigm of integration across traditional biological disciplines. The candidate will complement a faculty whose scale of research spans from molecules to landscapes and will contribute to a new undergraduate program in Molecular and Integrative Biology. Teaching duties will not exceed two courses per year during the first several years. Courses taught will include a course in the candidate's area of expertise and participation in a team-taught Introductory Biology course. The successful candidate will demonstrate the ability to establish a productive and extramurally funded research program and actively train M.S. and Ph.D. students. Position will begin 16 August 2011. A Ph.D. is required and postdoctoral experience is desirable. Review of applications will begin October 25 and continue until the position is filled. Send curriculum vitae, three representative reprints, statement of teaching and research interests, and names and contact information for three references to: **Dr. Turk Rhen (e-mail: turk.rhen@und.edu), 10 Cornell Street Stop 9019, University of North Dakota, Grand Forks, ND 58202-9019.** For more information visit **website: http://www.und.edu/dept/biology/jobs.htm.** *The University of North Dakota is an Equal Opportunity/Affirmative Action Employer and we strongly encourage applications from women and underrepresented groups.*

POSITIONS OPEN

UNIVERSITY OF NOTRE DAME

FACULTY OPENINGS
University of Notre Dame
Chemical and Biomolecular Engineering

The Department of Chemical and Biomolecular Engineering at the University of Notre Dame is pleased to announce openings for tenured or tenure-track faculty at any rank. A Ph.D. or equivalent degree is required. All research fields are welcome. Applicants should have developed, or show potential for development of, an outstanding research program and possess a strong commitment to graduate and undergraduate education. Applicants should send a PDF file of their curriculum vitae, statement of teaching and research interests, and the names and complete addresses of at least four references to **e-mail: cbeopen@nd.edu.** Please direct any questions to **e-mail: mjm@nd.edu.** *Notre Dame is an Equal Opportunity/Affirmative Action Employer.*

UNIVERSITY of OKLAHOMA
Department of Botany and Microbiology

Applications are invited for the tenure-track positions to begin August 2011, at the **ASSISTANT PROFESSOR** level. The Department seeks outstanding individuals who will contribute to its research, teaching, and service missions, with specific research interests in: (Search #1) Plant Molecular Biology by studying fundamental aspects of cellular and/or developmental biology with an emphasis in cell signaling, functional genomics, proteomics/metabolomics, cell biology, molecular evolution, or plant-microbial interactions. (Search #2) Plant Ecology by studying plant roles in the regulation of ecosystem structure and function in response to climate change, bioenergy production, or invasive plant ecology.

Candidates must have a Ph.D. in Botany or closely related discipline, strong record of research publication, postdoctoral experience, and provide evidence of a strong ability to develop independent, extramurally funded research. The successful candidate will also participate in graduate instruction and training and teach undergraduate courses that support the departmental curriculum. Applicants should indicate Search # and send hard copies of the following to: **Dr. Gordon Uno, Chair, Department of Botany and Microbiology, 770 Van Vleet Oval, University of Oklahoma, Norman, OK 73019** (inquiries to **e-mail: guno@ou.edu**): curriculum vitae, representative reprints, statements of research plans and teaching interests and philosophy, as well as full contact information for four references. Review of applications begins November 15, 2010, and will continue until positions are filled. More information about the faculty, Department, and searches may be obtained online at **website: http://www.ou.edu/cas/botany-micro/.** *Women and members of underrepresented groups are encouraged to apply. The University of Oklahoma is an Equal Opportunity Employer.*

FACULTY POSITION
Section of Endocrinology
Tulane School of Medicine

Tenure-track position, **ASSISTANT** or **ASSOCIATE PROFESSOR** level. Section focus is on diabetes. Funded candidates with molecular and/or clinical pathophysiology are encouraged to apply. Research and teaching responsibilities. Adjunct appointment(s) with physiology, biochemistry, pharmacology also possible. Section has a strong clinical research/clinical trials program. Clinical samples/materials available for collaborative clinical/translational research. Section also involved in epidemiology studies and a pilot program of clinical translation of stem cell therapy in diabetes. Curriculum vitae, research and teaching goals, three references to **website: http://vfonseca@tulane.edu**. Search open until qualified applicant is identified. *Affirmative Action/Equal Opportunity Employer. Women and minorities invited to apply.*

POSITIONS OPEN

BIOLOGY TENURE-TRACK POSITIONS
Department of Biology
Georgia Southern University

The Department of Biology invites applications for three tenure-track positions at the **ASSISTANT PROFESSOR** rank to begin 1 August 2011. The full text advertisement, including information about the department, faculty, and the complete position announcement with all qualifications and application instructions, is available at **website: http://www.bio.georgiasouthern.edu/searches.** Georgia Southern seeks to recruit individuals who are committed to excellence in teaching, scholarship, and professional service within the University and beyond and who are committed to working in diverse academic and professional communities. Research that can complement an integrative program exploring the biology of the Southeastern coastal plain is especially welcome. Deadline for postmarked applications is 22 October 2010. Applicants must have a Ph.D. by 31 December 2010.

Assistant Professor in **AGRICULTURAL/FORESTRY ECOLOGY.** We seek an individual using creative ecological approaches to address important applied problems in a landscape dominated by agriculture and silviculture. The successful candidate will contribute to teaching needs at the undergraduate and graduate levels.

Assistant Professor in **DEVELOPMENTAL BIOLOGY.** Applicants whose research focuses on animal developmental biology using an integrative or evolutionary approach are encouraged to apply. The successful candidate will teach undergraduate and graduate level courses including developmental biology.

Assistant Professor in **MICROBIAL ECOLOGY.** The successful candidate will perform research in microbial interactions or microbe-macrobe interactions, preferably in an applied context, and will teach undergraduate and graduate courses in Microbiology and related fields.

Applicants must submit hard copies of a letter of application, curriculum vitae, and statements of research and teaching interests. Applications must also include three letters of reference, which may be sent via e-mail in PDF format. Submit all materials to the appropriate search chair:

Agricultural/Forestry Ecology. **Dr. C. Ray Chandler,** chair, Search **#59285. E-mail: agroecologysearch@georgiasouthern.edu.**

Developmental Biology. **Dr. Sophie George,** chair, Search **#59282. E-mail: developmentalbiologysearch@georgiasouthern.edu.**

Microbial Ecology. **Dr. Dana Nayduch,** chair, Search **#59288. E-mail: microsearch@georgiasouthern.edu.**

Department of Biology, P.O. Box 8042. Georgia Southern University, Statesboro, GA. 30460. *Georgia Southern University is an Affirmative Action/Equal Opportunity Institution and the state is an Open Records state. Finalists will be required to submit to a background investigation. Individuals who need reasonable accommodations under the ADA to participate in the search process should contact the Associate Provost.*

A **POSTDOCTORAL RESEARCH** position is available in the Department of Pediatrics at Baylor College of Medicine to study molecular mechanisms of hyperoxic lung and retinal injury in the newborn. We are particularly interested in the mechanistic role of cytochrome P4501A enzymes in the prevention of chronic lung disease in newborn rodents and oxygen-induced retinopathy. We will use transgenic and knockout mouse models, and utilize state-of-the-art molecular techniques. Knowledge and skills in ophthalmology research and angiogenesis would be highly desirable.

Please send curriculum vitae to **Dr. Xanthi Couroucli** via **e-mail: xanthic@bcm.edu.**

PLANT BIOCHEMIST

The Department of Botany, University of Wisconsin-Madison (**www.botany.wisc.edu**) invites applications for an ASSISTANT PROFESSOR to begin as early as August 2011. Ph.D. required prior to start of appointment.

The individual who fills this position will be expected to establish an exciting, high-impact research program in the broad area of plant biochemistry. Candidates are sought who will add strength including but not limited to, chemical signaling, systems biology, metabolomics, chemical ecology, photosynthesis, bioenergy, or symbiosis. Teaching responsibilities would include contributions to an introductory biology class and/or graduate level plant biochemistry course.

Applicants should submit their curriculum vitae, and statements of research and teaching goals, and arrange for the delivery of three letters of recommendation. Application materials should be sent to: **Professor Simon Gilroy (sgilroy@wisc.edu), Search Committee Chair, Department of Botany, University of Wisconsin-Madison, 430 Lincoln Drive, Madison, WI 53706**. All materials must be received by **November 19, 2010,** for full consideration.

The University of Wisconsin is an Equal Opportunity Employer. We promote excellence through diversity and encourage all qualified individuals to apply. Unless confidentiality is requested in writing, information regarding applicants must be released upon request. A criminal background check may be required prior to employment.

ILLINOIS
UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

William J. Rutter Endowed Chair in Biochemistry
Department of Biochemistry
School of Molecular and Cellular Biology

The Department of Biochemistry (**http://mcb.illinois.edu/departments/biochemistry/**) at the University of Illinois at Urbana-Champaign seeks outstanding applicants for an endowed, tenured position at the rank of Professor. Candidates must hold a PhD, MD, or equivalent degree, and have a strong international reputation in an area of biochemistry. The target starting date is August 16, 2011. Successful candidates must conduct independent research, perform academic duties associated with our BS and PhD programs, and have the ability to teach effectively at both the graduate and undergraduate levels. Salary is competitive and commensurate with experience. This position offers excellent laboratory facilities, relocation startup funds, and the opportunity to work with outstanding colleagues and graduate students. The Department seeks to strengthen its efforts in diverse areas including, but not limited to: RNA biochemistry, membrane protein structure and function, drug target development and other areas of contemporary structural biochemistry. The successful candidate would also provide synergy with major campus research thrusts, particularly in cancer, neurobiology, stem cell and developmental biology, reproductive biology, immunology, cardiovascular biology, antibiotic development, or infectious disease. The Urbana-Champaign campus offers a wide range of state-of-the-art research support facilities that include the Roy J. Carver Biotechnology Center, the W. M. Keck Center for Comparative and Functional Genomics, as well as facilities for X-ray crystallography, NMR, EPR, proteomics, metabolomics, high-throughput screening, immunology, flow cytometry, microscopy, and transgenic mice. The campus has strong programs in biophysics, bioengineering and chemistry, and superb computational resources are available at the National Center for Supercomputing Applications and the NIH Resource for Macromolecular Modeling and Bioinformatics. The University of Illinois is a partner in the Life Sciences Collaborative Access team (LS-CAT) at nearby Argonne National Labs providing ready access to the synchrotron.

Urbana-Champaign offers the residential advantages of a medium-sized university city, excellent cultural opportunities, and a high quality of life as reflected by its ranking as one of 10 Great Neighborhoods in America by the American Planning Association: **http://www.planning.org/greatplaces/neighborhoods/2007/westurbana.htm**

To ensure full consideration, create your candidate profile through **https://jobs.illinois.edu** and upload your application cover letter, curriculum vitae, a concise summary of past research accomplishments, and a statement of future research plans, by **December 15, 2010**. Although early applications are appreciated and interviews may be conducted before the closing date, no hire will be made until after the closing date. Questions can be addressed to the School of Molecular and Cellular Biology, 217-333-3166.

The University of Illinois is an Affirmative Action/Equal Opportunity Employer. The administration, faculty, and staff embrace diversity and are committed to attracting qualified candidates who also embrace and value diversity and inclusivity.

Cincinnati Children's Research Foundation



Associate Directors

Cincinnati Children's Hospital Research Foundation of the Cincinnati Children's Hospital Medical Center is currently conducting a search for two positions, an Associate Director of Basic Science and a second Associate Director of Clinical Translational Outcomes and Health Services Research. These positions will lead the development and implementation of the Cincinnati Children's Research Foundation's strategic direction for research and training to improve the health of children.

Faculty members will hold academic appointments at the University of Cincinnati School of Medicine. The ideal scholarly candidate will hold an MD, PhD or combined MD - PhD, and should have a distinguished national and international reputation for research excellence. Candidate should be a recognized leader in his/her respective scientific field and have a demonstrated ability to teach at the university and medical school level, conduct research, mentor faculty, prepare grants, pursue external funding and publish in refereed journals. Experience should include working in an interdisciplinary medical setting involving a culturally diverse population.

Cincinnati Children's Hospital Research Foundation is one of the largest pediatric research programs in the nation, ranking second among all pediatric institutions in funding from the National Institutes of Health and sixth among all American hospitals. Cooperation and collaboration transcends all aspects of the institution, allowing us to meet our education, research and clinical care missions.

Interested candidates should send letter of interest along with CV to:
Jeffrey A. Whitsett, MD
Executive Director, Perinatal Institute
Chief, Section of Neonatology, Perinatal and Pulmonary Biology
3333 Burnet Avenue, MLC 7029
Cincinnati, Ohio 45229
jeffrey.whitsett@cchmc.org
Phone: 513-636-4830

Cincinnati Children's Hospital Medical Center is an Affirmative Action/Equal Opportunity Institution. Women and Minoritites are encouraged to apply.

Cincinnati Children's
change the outcome®

MBL Biological Discovery in Woods Hole

Director of NIH-NCRR Supported National *Xenopus* Resource

at the Marine Biological Laboratory, Woods Hole, MA

The MBL is seeking a Director for the recently established NIH-NCRR-supported National *Xenopus* Resource. In addition, the successful candidate will be expected to conduct externally funded, independent research within one of the Centers or Programs of the MBL and will be considered for appointment at the Assistant, Associate or Senior Scientist level depending on qualifications.

The Director will oversee the scientific and operational needs of the Resource, together with input and expertise contributed by an advisory committee* of *Xenopus* researchers from around the country. The Resource includes a newly renovated, purpose-built facility to support both *Xenopus laevis* and *Xenopus tropicalis*, and other resources including a P40 support grant from the NIH's National Center for Research Resources (NCRR). The goal of the National *Xenopus* Resource is to support basic and translational research using this model organism.

Applicants must have a Doctorate in Biology or closely related field, extensive experience with *Xenopus* as a model research organism, strong managerial and supervisory experience, a strong record of scientific publication, and the potential to attract extramural funding for their research.

Applicants should submit a CV, statement of interest in Directorship, separate statement of research interest, and list of four references on-line at: **https://mbl.simplehire.com.**

*Advisory Committee: Robert Grainger, University of Virginia (Chair); Richard Harland, University of California, Berkeley; Mustafa Khokha, Yale University; Kristen Kroll, Washington University; John Wallingford, University of Texas, Austin.

For more information about the job posting and the MBL please go to **www.mbl.edu**.

The MBL is an Equal Opportunity/Affirmative Action workplace.

POSITIONS OPEN

TENURE-TRACK FACULTY
Department of Computational and Systems Biology
University of Pittsburgh
School of Medicine

We invite applications for a full-time, tenure-track Faculty position at the **ASSISTANT, ASSOCIATE,** or **FULL PROFESSOR** level in the Department of Computational and Systems Biology in the School of Medicine at the University of Pittsburgh. Candidates with outstanding qualifications should demonstrate research accomplishment in computational and theoretical analysis, modeling and simulation of biological systems. Candidates with suitable expertise may have the possibility to establish a joint experimental and computational research program. In accordance with the multidisciplinary research activities and integrative role of the department, the appointee will have the unique opportunity to collaborate with Faculty from the School of Medicine and the Faculty of Arts and Sciences at the University of Pittsburgh, and with researchers from the Pittsburgh Supercomputing Center and Carnegie Mellon University. Qualified applicants are invited to send their curriculum vitae, a short summary of research interests and plans, and three reference names by December 1, 2010, to **e-mail: nmg5@pitt.edu** or to: **Faculty Search Committee, Department of Computational and Systems Biology, University of Pittsburgh School of Medicine, 3064 Biomedical Science Tower 3, 3501 Fifth Avenue, Pittsburgh, PA 15260, USA.** *The University of Pittsburgh is an Equal Opportunity/Affirmative Action Employer.*

CHEMICAL BIOLOGY

The Department of Chemistry at the University of Michigan invites applications for a tenure-track position at any rank with a proposed start date of September 1, 2011. Candidates with research interests in the area of chemical biology will be given priority. This would be a University-year appointment (nine-month academic salary with summer salary supported by research funds). Candidates are expected to develop an internationally recognized program of scholarly research and to excel in teaching at undergraduate and graduate levels. Detailed information regarding the electronic application process and required materials is available online at **website: https://www.chem.lsa.umich.edu/chem/facultyrecruit/**. The position will remain open until filled but preference will be given to applicants who have submitted all requested materials prior to October 15, 2010. Information about the Chemistry Department is available on the **website: http://www.umich.edu/~michchem.** Questions about the application process should be sent to **e-mail: chemfac10@umich.edu.** *Women and minorities are encouraged to apply. The University of Michigan is supportive of the needs of dual career couples and is an Equal Opportunity/Affirmative Action Employer.*

FACULTY POSITIONS

The Oregon Hearing Research Center (OHRC) invites applicants for faculty positions. Appropriate candidates will have research programs using molecular, cellular, or systems approaches to auditory and vestibular neuroscience. Junior-level applicants (including postdocs) are particularly encouraged to apply, although candidates at all levels will be considered. Faculty are expected to maintain an outstanding program of original research and to be an effective teacher at the graduate level. The OHRC, located in beautiful Portland, Oregon, is among the top auditory research centers in the USA, with faculty studying development, function, and disease of the inner ear and auditory central nervous system. Please send (by e-mail only) a detailed curriculum vitae, a brief description of research accomplishments and future plans, PDF copies of representative publications, and the names of three references to: **OHRC Search Committee, Attn: Sara Van Noy, e-mail: vannoys@ohsu.edu.** Consideration for completed applications will begin November 1, 2010.

POSITIONS OPEN

LOYOLA MEDICINE
Loyola University Chicago Stritch School of Medicine

TENURE-TRACK FACULTY POSITION

The Department of Molecular Pharmacology and Therapeutics at Loyola University Chicago, Stritch School of Medicine is recruiting a tenure-track faculty member at the rank of **ASSISTANT** or **ASSOCIATE PROFESSOR.** Candidates with established, productive research programs that complement existing programs within both the Department and the newly established Institute of Signal Transduction (IST) are especially encouraged to apply. Generous start-up funds and laboratory space are available. For more information about the department and the IST visit **websites: http://www.luhs.org/depts/pharmacology/index.html** and **http://www.stritch.luc.edu/signal_transduction/**, respectively. Applicants should have a Ph.D. and/or M.D. degree, and be committed to excellence in both research and the teaching of Pharmacology. Applications that include curriculum vitae, statement of research interests, and names of at least four references should be sent electronically to **e-mail: molpharmrecruit@lumc.edu.** *Equal Employment Opportunity/Affirmative Action Employer.*

ASSISTANT PROFESSOR in Plant Development and Evolution
Department of Plant Biology
University of Vermont

The Department of Plant Biology at the University of Vermont is seeking applications for a plant evolutionary developmental biologist to join a diverse group of active scholars in the department. The position is for a tenure-track Assistant Professor, beginning in the fall of 2011. The successful candidate will show evidence of ability to implement a productive research program, consistently attract extramural funding, and develop a strong teaching record. Teaching will be in introductory biology for majors as well as in an area of the candidate's expertise. A Ph.D. and postdoctoral in a relevant area are required. Preference will be given to candidates whose research addresses evolutionary explanations of plant form and/or function especially within the context of environmental signals. The University of Vermont recently identified three "Spires of Excellence" in which it will strategically focus institutional investments and growth over the next several years. One of these spires is Complex Systems (original proposal may be viewed at **website: http://www.uvm.edu/~ovpr/CSYS.pdf**). Candidates whose research, scholarship, and/or creative work interests align or intersect with Complex Systems are especially encouraged to apply.

Applicants should apply online at **website: http://www.uvmjobs.com** (**#033581**). A letter of application, curriculum vitae, a research statement, and a statement of interests and vision regarding teaching should all be attached electronically to the online application. In addition, applicants should enter in the application names and e-mail addresses for three individuals who will provide letters of reference. The University is especially interested in candidates who can contribute to the diversity and excellence of the academic community through their research, teaching, and/or service. Applicants are requested to include in their cover letter information about how they will further this goal. Review of applications will begin on October 1, 2010, and will continue until the position is filled. Questions may be directed to **Dr. Jeanne Harris,** search chair, at **e-mail: jeanne.harris@uvm.edu.**

The University of Vermont is an Affirmative Action/Equal Opportunity Employer. The Department of Plant Biology is committed to increasing faculty diversity and welcomes applications from women and underrepresented ethnic, racial, and cultural groups and from people with disabilities.

POSITIONS OPEN

The Department of Neurobiology and Anatomy at Wake Forest University School of Medicine (WFUSM) invites applications for a **FACULTY POSITION** (rank open) from investigators with outstanding, multidisciplinary research programs utilizing molecular, cellular, and functional approaches including behavioral assessments to study mechanisms of neuronal injury and repair or neurodegeneration in any suitable experimental model system. The individual appointed for this position will join an active faculty whose research investigates synaptic plasticity, regulation of Ca2+ channels, neuronal activity and cellular and molecular changes associated with CNS development, aging, multisensory integration, epilepsy, and motor neuron diseases. WFUSM has a large and vibrant neuroscience community with strong tradition of collegiality and collaboration, and the successful candidate will be expected to participate in translational research by promoting collaborations with clinicians with shared interests and expertise. The position will be available July 1, 2011. Candidates should send curriculum vitae, statement of specific research interest, and three letters of recommendation to: **Search Committee, Department of Neurobiology and Anatomy; Wake Forest University School of Medicine, Winston-Salem, NC 27157-1010.** For more information on the Department and areas of research emphasis, visit us online at **website: http://www.wfubmc.edu/nba.** *Wake Forest University Baptist Medical, and its component institutions, is an Affirmative Action and Equal Opportunity Employer with a strong commitment to achieving diversity among its faculty and staff.*

POSTDOCTORAL POSITION

Department of Biological Sciences, College of Science, Marshall University, Huntington, West Virginia. Position available starting November 1, 2010, for a highly motivated postdoctoral fellow to pursue studies on the effects of diet-mediated epigenetic modifications on cancer. Exciting research environment, state-of-the-art equipment and facilities are available (**website: http://musom.marshall.edu/research/**). Mandatory requirements: Ph.D. in molecular biology or related field, a track record of publications, excellent computer skills (for bioinformatics analysis), and good oral and writing skills. Expertise in epigenetics (ChIP/chip, ChIP-Seq, chromatin remodeling assays), cell culture, biochemical and standard molecular assays required. Other expectations: presentation of research findings at scientific conferences, preparation of manuscripts, and applications for external postdoctoral funding. To apply (PDF files preferred), submit a statement of research interests, curriculum vitae, and a brief description of career goals, as well as the names, e-mail addresses, and telephone numbers of at least three references to: **Philippe Georgel, Ph.D., Byrd Biotechnology Science Center, Room 241-P, 1700 Third Avenue, Huntington, WV 25755 USA; e-mail: georgel@marshall.edu.** Review of applications begins on October 1, 2010.

ASSISTANT PROFESSOR in Eukaryotic Molecular Biology

Biology Department of Indiana University Southeast seeks qualified Ph.D. applicants for tenure-track position beginning August 2011. Postdoctoral and teaching experience preferred. Teaching duties include Molecular Biology, Introductory Biology, and related courses. For more information and directions on how to apply visit **website: http://www.ius.edu/hr/.** Deadline October 29, 2010. *IUS is an Affirmative Action/Equal Opportunity Employer.*

University of Colorado
School of Medicine

The Department of Biochemistry and Molecular Genetics at the University of Colorado School of Medicine in Denver invites applications for **Assistant Professor** faculty positions. We are broadly interested in candidates focusing in the areas of **biophysics or genomics or biochemistry**. Successful candidates will be expected to establish a vigorous, innovative and independent research program and participate in teaching. They will join a highly interactive, interdisciplinary group of faculty (**http://www.uchsc.edu/sm/bbgn/**), students, and fellows, and enjoy access to state-of-the-art equipment and facilities on our new campus. Candidates must hold a PhD (or equivalent) degree and have a strong record of research accomplishments.

Applicants should submit via **jobsatcu.com**, a CV, a brief description of research accomplishments and plans, and names of at least three people who can write a letter of reference.

We will begin reviewing applications **November 15, 2010**.

iPierian

Senior Scientist and Senior Staff Scientist Assay Development and Lead Discovery

iPierian is a pioneering biopharmaceutical company that is taking the cutting-edge technologies of cellular reprogramming and directed differentiation to an entirely new level to harness the power of induced pluripotent stem cells to advance the understanding of human diseases and accelerate the discovery of more effective therapeutics for patients. iPierian is dedicated to making iPSC technology the industry standard for the early introduction of human pharmacology into the drug discovery process.

A position at iPierian is an opportunity to join a team of leading scientists and collaborators committed to improving human health. We hire outstanding people and subscribe to a rigorous, fast-paced work environment where the science leads the business. If you are interested in joining the iPierian team, please submit your resume/CV online at **careers@ipierian.com** and reference SC01 when applying.

The successful candidate will be responsible for the design, development, and execution of medium and high throughput cell-based assays in a variety of formats and screening modalities to support iPierian's therapeutic programs. She/he will work closely with colleagues, within a matrix organization, on projects ranging from early to late stage discovery. This individual will be capable of integrating the highest quality assay and automation technologies to advance the drug discovery process. The individual must be capable of taking full responsibility of building an assay "from scratch" suitable for high volume screens, lead optimization and molecular target validation. This individual should be able to balance hands-on laboratory work with managerial responsibilities and may manage external collaborations. Salary will be commensurate with experience.

Required Qualifications:

- Ph.D. or equivalent degree
- At least 4-8 years of industrial or academic experience in planning, development and execution of assays and running medium to high throughput screens for small molecule drug discovery
- Minimum 2 years of supervisory experience
- Experience in developing and optimizing novel assays in a variety of conventional and cutting edge formats and modalities including cell-based assays in complex cell cultures and biochemical assays focused on specific molecular targets, is required.
- Extensive experience in scaling up and qualifying assays for large scale screens is required
- Demonstrated research experience in neurobiology is preferred
- Ability to conduct a project that requires outsourcing of assay development and screening activities
- Excellent verbal and written skills are required
- Demonstrated ability to work in a fast paced multidisciplinary team

Tenure-Track Faculty Positions

The Department of Biochemistry and Molecular Biology of The University of Texas MD Anderson Cancer Center seeks an outstanding basic research scientist, at the level of Assistant or Associate Professor, with recognized expertise in stem cell and developmental biology. We are particularly interested in candidates whose interests align with departmental strengths in developmental biology, epigenetics, signal transduction and cancer biology. The successful applicant must hold a Ph.D. and/ or an M.D. degree, and is expected to develop an internationally recognized, extramurally funded research program and be active in graduate student training. Outstanding candidates will receive a generous start-up package, supplemented by the Center for Stem Cell and Developmental Biology and targeted funding by the Cancer Prevention and Research Institute of Texas and the MD Anderson Research Trust. To apply, please send, within a SINGLE PDF, your Cover Letter, CV and Short Research Summary (three-page maximum). In addition, three confidential reference letters should be submitted, preferably in PDF format.

All application materials should be sent by e-mail to: **stemcellsearch@mdanderson.org; Department of Biochemistry & Molecular Biology, UT MD Anderson Cancer Center, 1515 Holcombe Blvd. Unit 1000, Houston, TX 77030**.

Receipt Deadline: **November 15, 2010**

THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center

MD Anderson Cancer Center is an equal opportunity employer and does not discriminate on the basis of race, color, national origin, gender, sexual orientation, age, religion, disability or veteran status, except where such distinction is required by law. All positions at MD Anderson are considered security sensitive; drug screening and thorough background checks will be conducted. The University of Texas MD Anderson Cancer Center values diversity in its broadest sense. MD Anderson is a smoke-free environment.

Division of Human Genetics/Integrative Genomics Center



Full/Associate/ Assistant Professor

The Division of Human Genetics/Integrative Genomics Center of the Cincinnati Children's Hospital Medical Center, led by Dr. Gregory Grabowski, is undergoing major expansion to meet our long-term goals to improve child and adult health through excellence in genetic research, translational programs, clinical practice and education. Outstanding Faculty Physician (M.D., D.O., M.D./Ph.D.) and Ph.D. Scientists will have appointments commensurate with experience and accomplishment. The large populations in our general and specialized clinics, as well as our reputation as a world-class medical institution, offer extraordinary clinical and basic research opportunities. Highly competitive start-up support is available to develop research and clinical programs that foster career development.

Areas of recruiting interest for established and developing faculty are: **Translational Research, Molecular and Personalized Medicine, Diagnosis and treatment of genetic disorders and Quantitative and Integrative Genomics – Applied and Basic**.

Each focus represents a critical area promoting direct opportunities to integrate research into patient care. These exceptional opportunities, in combination with Cincinnati Children's strong, multidisciplinary infrastructure and our commitment to faculty support, make our institution highly attractive to launch and/or expand your career and change health outcomes for children and adults. Cincinnati Children's supports disease-based programs across the continuum of research to move basic laboratory/population discoveries into translational programs that lead to patient-oriented studies and clinical trials for new treatments.

At Cincinnati Children's Hospital Medical Center, we are committed to improving the quality of life for children and adults, locally and worldwide. Cincinnati Children's Hospital Medical Center is one of only eight pediatric hospitals in the United States to be included on the Honor Roll in *U.S. News & World Report*'s 2010 Best Children's Hospitals issue. Among pediatric institutions, Cincinnati Children's Hospital Medical Center is the second-highest ranking recipient of research grants from the National Institutes of Health.

Interested candidates should send their CV, Bibliography (including funding record) and letter of interest to:
Julie Burns, Physician and Faculty Recruitment
Email: julie.burns@cchmc.org
Phone: 513-636-5260

Please visit our career page at **www.cincinnatichildrens.org (job id 39789)**.

Cincinnati Children's Hospital Medical Center is an Affirmative Action/Equal Opportunity Institution. Women and Minorities are encouraged to apply.

Cincinnati Children's
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POSITIONS OPEN

FACULTY POSITION
University of Missouri School of Medicine
Department of Nutrition and Exercise Physiology

The Department of Nutrition and Exercise Physiology, University of Missouri School of Medicine invites applications for a tenured or tenure-track position at any rank with preference given to applicants at the rank of **ASSISTANT** or **ASSOCIATE PROFESSOR**. We are interested in an investigator who primarily conducts human or translational nutrition and metabolism research in the area of obesity and its associated behaviors and/or complications (type 2 diabetes, metabolic syndrome, inflammation, etc). The newly expanded Department of Nutrition and Exercise Physiology now spans three colleges within the University providing a unique opportunity for the department to utilize resources in the Medical School and on the main campus. Position qualifications include a Ph.D., M.D., or a M.D.-Ph.D. with postdoctoral experience. Successful applicants will have or develop an outstanding research program and contribute to School of Medicine teaching activities. Involvement in campus-wide research initiatives relative to obesity, cardiovascular biology, exercise, metabolism, cell signaling, and nutrition is desirable. Located midway between St. Louis and Kansas City, Columbia is a vibrant university town that is consistently ranked among the top small cities to live in America.

Please send curriculum vitae, a narrative of research and educational interests, and the names and contact information of three references to:

Catherine Peterson, Ph.D.
Chair, Nutrition Search Committee
Department of Nutrition and Exercise Physiology
School of Medicine/HES/CAFNR
217 Gwynn Hall
University of Missouri-Columbia
Columbia, MO 65211
Or, by electronic submission (strongly preferred)
To e-mail: umchesnsjobs@missouri.edu

Active review of applications will begin November 1, 2010, and the search will continue until the position is filled. *The University of Missouri is very interested in promoting a diverse work environment and has programs in place to promote diversity on campus. The University of Missouri is an Equal Opportunity/Affirmative Action Employer, and complies with the guidelines set forth in the Americans with Disabilities Act of 1990.* Visit the University of Missouri-Columbia online at **website: http//www.missouri.edu/**. Please direct ADA accommodation requests to our coordinator at **telephone: 573-884-7278** (V/TTY)

TENURE-TRACK POSITION in Chronic Human Disease

The Department of Biochemistry at Virginia Tech seeks applicants for a tenure-track **ASSISTANT PROFESSOR** position in the area of obesity and other chronic human diseases. Exceptionally qualified applicants for higher ranks will also be considered. Candidates who apply contemporary metabolomic, proteomic, or genomic approaches to understanding the nature and impact of metabolic anomalies associated with obesity, diabetes, heart disease and their comorbidities will be considered. The successful candidate will build and maintain an internationally recognized extramurally funded research program, will participate in instruction, and will be open to collaborative research. Competitive startup packages will be provided. The successful candidate will join a group of active and collaborative colleagues in obesity and other chronic human disease research. Applicants must apply online (**website: https://listings.jobs.vt.edu,** use posting number **0100659**) and arrange for three reference letters to be sent electronically, if possible, to one of the co-chairs of the Chronic Human Disease Search Committee, **Dr. David Bevan** (**e-mail: drbevan@vt.edu, telephone: 540-231-5040**) and **Dr. Jianyong Li** (**e-mail: lij@vt.edu, telephone: 540-231-1182**), **Department of Biochemistry, Virginia Tech, Blacksburg, VA 24061.** Applications will be reviewed beginning October 8, 2010. However, the position will remain open until filled. For complete job description and departmental information, see **website: http://www.biochem.vt.edu.**

POSITIONS OPEN

The Ohio State University Colleges of Medicine and Public Health announce a unique cross-college tenure-track/tenure eligible **FACULTY POSITION** in infectious diseases with emphasis on clinical trials/investigations (**website: http://www.phpid.osu.edu**). *OSU is an Equal Opportunity Employer.*

EYEGATE PHARMACEUTICALS, INC., a Waltham-based specialty pharmaceutical company seeks **PRESIDENT/CEO.** Responsibilities include: analyze project operations; identify new product opportunities and conduct private/public fundraising; promote company to both private and public investors and to merger/acquisition candidates. Minimum requirements are Master's degree (or equivalent) in Accounting or Finance. Employer will also accept Bachelor's degree (or foreign equivalent) in Accounting or Finance plus five years of progressively more responsible work in the field; and four years of senior level investment/banking experience with companies in the life sciences, health and biotechnology/medical device industries. Experience must include: qualified Accounting experience in multinational (Fortune 500) accounting firm; U.S. and European corporate finance experience in taking companies public; raising and managing $10 million plus in venture capital/private funding; M&A transactions; experience as senior executive/officer of a life science company; scientific/technical understanding of biotechnology/pharmaceutical/life science industry; franchise building; budget planning/analysis. Please send cover letter and resume to: **Paul Perkins, Eyegate Pharmaceuticals, Inc., 100 Beaver Street, Waltham, MA 02453.**

GENERAL ADVERTISEMENT

The Department of Chemistry at the University of Michigan invites applications for an anticipated **TENURE-TRACK POSITION** at any rank in any subdiscipline of Chemistry with a proposed start date of September 1, 2011. This would be a University-year appointment (nine-month academic salary with summer salary supported by research funds). Candidates are expected to develop an internationally recognized program of scholarly research and to excel in teaching at undergraduate and graduate levels. Detailed information regarding the electronic application process and required materials is available online at (**website: https://www.chem.lsa.umich.edu/chem/facultyrecruit/**). The position will remain open until filled but preference will be given to applicants who have submitted all requested materials prior to October 15, 2010. Information about the Chemistry Department is available on the **website: http://www.umich.edu/~michchem.** Questions about the application process should be sent to **e-mail: chemfac10@umich.edu.** *Women and minorities are encouraged to apply. The University of Michigan is supportive of the needs of dual career couples and is an Equal Opportunity/Affirmative Action Employer.*

POSTDOCTORAL POSITION
Theoretical Physics

Enrico Fermi Institute (EFI) seeks candidates for a **SIMONS POSTDOCTORAL FELLOWSHIP** at the University of Chicago with three year tenure and a salary of $65,000 per year, plus additional benefits. Applicants can be of any nationality. Applicants must have received their Ph.D. in some theoretical area in the year prior to the start of the Fellowship. Applicants can propose work in any field of EFI research; these include astrophysics, particle physics, atomic physics, relativity, and astronomy.

Complete applications should be forwarded electronically to **e-mail: l-kadanoff@uchicago.edu** before December 1. A complete application will include:

(1) An application and research plan,

(2) Three letters of recommendation from outside the EFI,

(3) References to and/or copies of recent papers.

A successful candidate will be selected on the basis of promise as a physical/mathematical scientist, ability to profit from and add to EFI, and potential for broad participation in EFI activities.

POSITIONS OPEN

EARTH and ENVIRONMENTAL SCIENCE
Professorship in Earth and Environmental Science

The Department of Earth and Environmental Science at the University of Pennsylvania invites applications for a tenured professorship in earth and environmental science that is expected to be filled at the **ASSOCIATE** or **FULL PROFESSOR** level. The research and teaching interests of the successful candidate must broaden and complement the Department's current activities in the areas of earth history and surficial processes. The successful candidate is expected to have developed an internationally recognized, externally funded, multidisciplinary research program and will be required to actively participate in undergraduate and graduate teaching and in the administration of the Department. Individuals who can further increase interactions with other University science departments are strongly encouraged to apply. Further information about the Department may be sought at **website: http://www.sas.upenn.edu/earth/.** Applicants apply online at **website: http://facultysearches.provost.upenn.edu/applicants/Central?quickFind=50821** with a cover letter, curriculum vitae, statements of research and teaching interests and, five publications. The Search Committee will begin to evaluate applications on November 10, 2010. The search will remain open until the position is filled. *The University of Pennsylvania is an Affirmative Action/Equal Opportunity Employer. Women and minority candidates are strongly encouraged to apply.*

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